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UREA, LYSINE, AND METHIONINE IN SWINE DIETS

by

© RAYMOND EINAR GRIMSON

A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES

IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE

OF MASTER OF SCIENCE

DEPARTMENT OF ANIMAL SCIENCE

EDMONTON, ALBERTA

FALL, 1969

THE UNIVERSITY OF ALBERTA

UREA, LYSINE, AND NITROGENOUS

OF BROOD DIETS

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 RAYMOND ERNAZ OLSINSKY

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The undersigned certify that they have read, and recommend to the faculty of Graduate Studies for acceptance, a thesis entitled "Urea, Lysine, and Methionine in Swine Diets" submitted by Raymond Einar Grimson, B. Sc., in partial fulfilment of the requirements for the degree of Master of Science.

ABSTRACT

These experiments were designed to evaluate urea as a source of nitrogen for swine fed diets supplemented with L-lysine and DL-methionine. In starting pig diets, 0.38% L-lysine or 0.38% L-lysine plus 0.12% DL-methionine was added; growing-finishing pig diets containing urea were supplemented with 0.42% L-lysine, or 0.42% L-lysine plus 0.12% DL-methionine. Diets not supplemented with urea, for growing-finishing pigs, contained only half this level of L-lysine. Experiments 1, 2, and 4 were factorially designed (2 x 3 x 2) with urea, amino acid combination, and sex as factors. Experiment 3 was designed as a 2 x 7 factorial with sex and seven dietary treatments as factors, in two replicates. Experiment 5, a metabolism study, was a 2 x 3 x 2 x 2 factorial in two replicates with the factors of urea, amino acid combinations, sex, and bodyweight (25 or 65 kg). Experiment 6 was a preliminary study of the nitrogen excretion pattern of young pigs fed a high level of dietary urea. Experiment 7, a N^{15}-urea study, was subjected to analysis in a one-way classification of two treatments in two replicates.

Isonitrogenous substitution of urea for fishmeal depressed feed intake, daily gain, and feed conversion in Experiment 1 but had no effects in Experiments 2 and 3. Growing and finishing pigs exhibited reduced daily gain and feed consumption although feed conversion was not affected. Additions of urea to the diet reduced the cross-sectional area of the longissimus dorsi (loin area), lean as a percentage of the ham face, and Record of Performance score. Inclusion of urea in the

diet increased nitrogen digestibility but had no influence on nitrogen retention and energy metabolism.

Addition of 0.38% L-lysine improved feed conversion of the pigs in Experiment 1 but had no influence on performance in Experiment 2. The feed conversion of pigs fed a low-protein diet in Experiment 3 was improved when L-lysine was added. A significant urea by lysine interaction affected daily gain and feed consumption in the growing and finishing swine in that these were improved when urea containing diets were supplemented with this amino acid. Carcass and growth measurements were unaffected by amino acid additions in Experiment 4.

Combined addition of 0.38% L-lysine plus 0.12% DL-methionine did not improve performance more than addition of L-lysine alone. Addition of the two amino acids reduced the retention of nitrogen. Digestible nitrogen coefficients were lowered when urea, L-lysine, and DL-methionine were added to the diets of the heavier pigs.

Metabolism of energy and nitrogen was influenced by bodyweight. At 25 kg bodyweight, the pigs digested and retained more of their gross energy consumption than at 65 kg bodyweight; however, at 65 kg bodyweight, they were more efficient than at 25 kg bodyweight with respect to retention of digestible energy. Moreover, the coefficient of nitrogen digestibility was higher at 25 kg than at 65 kg bodyweight.

Sex did not influence daily gain, feed consumption, or feed conversion of starting pigs. Growing-finishing barrows gained more rapidly, consumed more feed, and produced less desirable carcasses than gilts. In Experiment 5, the barrows digested and retained less energy and less nitrogen than the gilts.

The N^{15} of dietary N^{15}-urea was incorporated into the trichloroacetic acid-insoluble fractions of liver, blood plasma, intestinal mucosa, skeletal muscle, and blood cells. Blood plasma and liver were enriched to the greatest extent.

Excretion of N^{15} in the urine during 48 hr following administration of N^{15}-urea accounted for 52.2% of the administered dose; excretion during the subsequent 48 hr accounted for 1.9% of administered N^{15}. The results of the N^{15} experiment showed that dietary N^{15}-urea was used in body protein synthesis by the pig.

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INTRODUCTION

The potential utilization of urea for swine feeding offers an inexpensive alternative to existing protein supplies. However, many factors limit the efficiency with which the pig can convert the nitrogen of urea to a component of body tissue protein.

Studies with rats show that severe restriction of non-essential amino acid nitrogen is necessary to actuate the use of dietary urea nitrogen for protein synthesis. Nevertheless, the essential amino acids required by the animal must be supplied in accordance with their need. Because of these considerations, it is difficult to formulate diets of a practical nature that sufficiently restrict non-essential amino nitrogen intake and meet minimal requirements with respect to all of the essential amino acids. Furthermore, use of purified sources to supply all of the essential amino acids is not economical due to the prohibitive cost of some of these compounds. However, as synthetic amino acids become less expensive, it may be possible to design purified swine diets in which all the non-essential nitrogen is supplied as non-protein nitrogen and all the essential amino acids are supplied in synthetic form.

Experiments were undertaken at the University of Alberta during 1968 and 1969 to explore some of the effects of urea in swine diets. Also, it was desired to examine the influence of lysine and methionine additions to swine diets containing urea.

LITERATURE REVIEW

The utilization of non-protein nitrogen (NPN) compounds by ruminants is well documented. Of the many forms of NPN, perhaps the most widely studied has been urea which has been shown to effectively supply nitrogen (N) for bacterial protein synthesis in the rumen.

Urea is hydrolized in the rumen by bacterial urease releasing ammonia and carbon dioxide. Ammoniacal N is attached to intermediates of carbohydrate degradation to produce amino acids and eventually, bacterial protein.

Monogastric species are less adequately equipped to utilize dietary NPN compounds because microbial fermentation is poorly adapted to this type of digestive tract. There have been, nevertheless, indications in the literature that monogastrics such as the rat, pig, human, and chick may under certain conditions be able to use effectively some NPN as a non-essential N source in the synthesis of amino acids.

A review of the literature considering several aspects of the utilization of NPN by monogastric species, and especially by the pig, is presented.

Urea hydrolysis in the gastrointestinal tract

Nitrogen metabolism in the mammalian organism has been studied for many years, resulting in numerous reports concerning the fate of NPN compounds in monogastric species. As early as 1913, Taylor and Ringer reported that starved dogs retained in excess of 50% of the N of ammonium carbonate administered *per os* and thereby restored N balance. Kriss and Marcy (1940) reported that mature male rats receiving 2 g

urea daily in addition to an 8 g casein basal allowance excreted all but 3.8% of ingested urea. Studies involving human subjects have corresponded with these earlier findings. Urea, administered for diuretic purposes to humans, was never fully recovered (Moore et al., 1931). In a series of four experiments with adult humans, significant quantities of administered urea were not recovered in the urine or feces nor was the unrecovered urea accounted for by increases in blood NPN. Similar results were reported by Walser and Bodenlos (1959) who found that approximately 25% of intravenously administered C^{14} or N^{15}-urea was not recovered in the urine of healthy human subjects.

Studies using C^{14}-urea (Leifer, Roth, and Hempelman, 1948; Zbarsky and Wright, 1953; Chao and Tarver, 1953; Visek, Baron, and Switz, 1959; Levenson et al., 1959) indicated that up to 40% of administered C^{14} was expired as $C^{14}O_2$ within 24 hours after administration to mice and rats. Kornberg, Davies, and Wood (1954a, 1954b) have shown that in cats not secreting gastric juice, urea was catabolized at the rate of 0.2 to 0.5% per hour and suggest urease, catalyzing the reaction, is of microbial origin.

Although he reported slow or negligible hydrolysis of urea in rats, Bloch (1946) is supported in his supposition that gastrointestinal urease is of bacterial origin (Chao and Tarver, 1953; Kornberg et al., 1954a, 1954b; Kornberg and Davies, 1956; Levenson et al., 1959; Visek, 1962, 1964), that it is concentrated at the surface of the intestinal mucosa, and that it is responsible for the catabolism of urea to NH_3 and CO_2 in monogastric species. Conway et al. (1959), however, concluded the converse when they isolated urease using micro-

puncture techniques from within the gastric mucosal layer of a mouse.

Urease activity has been noted in the naso-pharyngeal and esophageal regions of cats (Kornberg et al., 1954a, 1954b; Kornberg and Davies, 1956) and in the stomach at sites protected from inactivation of the enzyme by acid.

Seneca, Peer, and Nally (1962) in their classification of microorganisms with respect to urea-splitting activity, describe urease-producing bacteria as either largely resistant to antibiotics or as those that can readily mutate to form resistant strains. However, antibiotics and certain chemicals have been demonstrated to slow or halt urea hydrolysis in the monogastric (cat, pig, mouse, rat, chick, and human) primarily by their effect on the bacterial population of the gastrointestinal tract (Dintzis and Hastings, 1953; Kornberg et al., 1954b; Walser and Bodenlos, 1959; Visek et al., 1959; Visek et al., 1961; Giordano, 1963; Harbers et al., 1963; Alvares, Hendrickson, and Visek, 1964; Visek et al., 1965).

Visek et al. (1959) tested the theory that antibiotics, at less than therapeutic levels, exert their growth promotant effect by: reduction of bacterial activity resulting in decreased intestinal ureolysis due to decreased microbial urease production; subsequent diminuition of the level of circulating ammonia (NH_3); resulting, thereby, in reduction of the energy required for the reformation of urea. They were unable, however, to demonstrate improvements in average daily gain (ADG), feed conversion (FC), or feed intake of rats fed low levels of antibacterial agent and administered subcutaneously with 1 ml quantities of C^{14}-urea. Expired $C^{14}O_2$ was reduced by the presence of

100 ppm of antibacterial agent, signifying reduced ureolysis, but this reduction was not accompanied by improvements in performance. Subsequent trials (Visek, 1962; Visek et al., 1965), however, have confirmed the hypothesis that reduction of ureolytic activity in the gastrointestinal tract of rats and chicks was accompanied by improved ADG and FC. Visek et al. (1965) reported that an interaction ($P < 0.01$) between 1% of dietary urea and 100 mg/kg bodyweight of chlorotetracycline occurred when these were fed in combination to rats. Whereas the addition of 1% of urea depressed ADG, chlorotetracycline addition with urea tended to abolish the depression in ADG produced by urea alone. Hays et al. (1957), however, did not find that the performance of 10.3 kg pigs (fed up to 40% protein-equivalent as urea) was significantly affected by the inclusion of dietary antibiotic.

Hydrolysis of exogenous or endogenous urea in the monogastric can be inhibited by immunization with urease of plant origin (generally purified crystalline jackbean urease) as reported by Dang and Visek (1960); Visek and Dang (1960); Visek (1962); Harbers et al. (1963b); Harbers, Hendrickson, and Visek (1963). These workers observed reduction of blood and intestinal NH_3 concentrations and concomitant improvements in the ADG and FC of rats and chicks. In dogs (Handford, 1961) and swine (Kornegay, Miller, and Hoefer, 1965a), urease administered intravenously at a level of 15 mg/kg bodyweight or injected intraperitoneally at a level of 110, 165, or 220 Sumner units (SU) of urease activity/kg of bodyweight, respectively, caused elevation of blood NH_3 level and reduced plasma urea level. Studies conducted by Kornegay et al. (1964) did not reveal significant improvements in ADG or FC when serial urease

injections of either 0, 0.22, 1.10, or 11.0 SU/kg bodyweight were administered to growing pigs fed diets containing 1.25, 2.5, and 3.4% urea. Immunization using these dosages, injected during a period of 47 days, did not significantly affect urea utilization by the pig. Wagner et al. (1963), in studies with chicks and rats, noted that dietary urease (20 to 61.5 SU) did not significantly affect rate or efficiency of gain. Similar experiments with Yorkshire x Poland China barrow pigs (initial weight 11.8 kg and 30.5 kg) by Glimp and Tillman (1964) indicated that jackbean urease immunization (total of 100 SU or 300 SU, injected intraperitoneally) did not increase serum titre nor improve ADG or FC; however, ADG and FC were improved ($P < 0.01$) by inclusion of 100 ppm of chlorotetracycline in the diet.

The origin, action, and inhibition of urease activity in the monogastric has been comprehensively reviewed and discussed by Kornberg and Davies (1955) and Visek (1964), respectively.

Addition of urea and other NPN compounds to the diets of pigs and other monogastric species

Effects of the level of NPN in the diet

Early research (cited by Hoefer, 1967) indicated that up to 40% of the pig's dietary protein could be replaced by urea without deleterious effects on the performance or health of the animals. Based on the more recent literature, Hoefer suggested that urea has little toxic effect when it supplies less than 30% protein equivalent in the ration. Hanson and Ferrin (1955) reported that urea, included at levels of 1.5 and 1.0% in the diets of growing and finishing swine, respectively, was not toxic and did not affect acceptability of the diet. Hays et al. (1957) fed

growing swine diets containing 8 and 10% total crude protein in which 0, 2.5, 5.0, 10.0, 20.0, and 40.0% protein equivalent was supplied as urea. Levels of 20 and 40% protein equivalent caused a depression in feed intake and markedly reduced the performance of the pigs. Diets containing 1.25% urea (Kornegay et al., 1964) did not significantly decrease ADG of starting pigs except when more than 35% of the total dietary N as soybean meal was replaced by urea. Subsequent trials conducted by Kornegay et al. (1965b) indicated that urea included in diets at a level of 2.5% initially suppressed ADG followed by subsequent improvements, indicating a tendency of the pig to adjust to dietary urea. Bowland (1967) reported that isonitrogenous replacement of fishmeal or soybean meal N by 2 or 3% of urea in rations not supplemented with the synthetic essential amino acids, L-lysine and DL-methionine, caused a depression in growth. Feed intake, however, was not affected. Urea included in diets at 1%, isonitrogenously replacing fishmeal or soybean meal, did not produce results similar to those obtained at higher levels of urea. Kornegay, Mosanghini, and Snee (1966) suggested that 1% urea or 15.5% of total N as urea in swine diets approached an optimum level as long as provision was made for replacement of essential amino acids lost by removal of protein from the diet.

Effects of urea and other NPN sources on feed intake

Daily feed intake was generally reduced as a result of inclusion of dietary urea (Hoefer, 1967), especially at levels in which urea supplied in excess of 15 to 20% protein equivalent. This agrees with other reports (Hanson and Ferrin, 1955; Hays et al., 1957; Kornegay et al., 1964; Kornegay et al., 1966). In earlier studies at the

University of Alberta, however, Bowland and Grimson (1969) observed no depression in feed intake when up to 3% urea was fed, a level equivalent to approximately 30% of the total crude protein equivalent.

Effects of urea and other NPN sources on average daily gain

Urea depressed ADG when Braude and Foot (1942) fed 17.4% protein equivalent diets such that 20 g of urea were consumed daily by growing and finishing swine. Urea had no positive effects on gain even when added to diets so low in protein that growth was restricted. Hanson and Ferrin (1955) found that addition of 1.5% of urea to low protein (10.6%) diets fed to 8-week-old pigs did not improve ADG to 56.8 kg liveweight as compared to the unsupplemented control pigs. Addition of 1.0% urea during the finishing period did not affect ADG indicating that 10.6% protein may have been adequate for optimum performance.

Although Hays et al. (1957) reported a slight improvement in the ADG of 10.3 kg pigs fed corn-soybean meal diets containing 16% protein and supplemented with 0.16 or 0.31% urea, additions of urea in amounts greater than 20.0% protein equivalent markedly reduced ADG. Similarly, other researchers have reported that urea fed to growing pigs at levels of 1.25, 2.5, and 3.4% of 17% total crude protein corn-soybean meal diets (Kornegay et al., 1964, 1965b); or, isonitrogenous substitution of 2 or 3% urea for fishmeal or soybean meal (Bowland, 1967) in the diets of 3-week-old weanling pigs tended to depress ADG. Kornegay (1966) and Kornegay et al. (1966), in substituting up to 2% urea for soybean meal or corn N in the diets of swine, noted a reduction in ADG: a greater depression resulted from urea replacement of soybean meal N than of corn N. As Hoefer (1967) suggested, it appears that urea exerts

a negative or neutral effect on ADG and that ". . . . no real evidence of significant improvement in rate of gain that could be attributed to the feeding of urea" is present in the literature.

Other species, such as chicks (Bice and Dean, 1942), exhibited an increasing depression of ADG when one-third, two-thirds, or all of the protein supplement was isonitrogenously replaced with urea in 16% protein diets. Weanling and mature male rats and 8-week-old pullets were used in studying NPN utilization by Berry, Riggs, and Kunkel (1956). Levels of 0.2, 0.4, 0.6, 0.8, and 1.0% biuret fed for 140 days did not significantly reduce the ADG of rats. In chickens, replacement of 1% of milo with 1.0% of urea, biuret, or soybean meal, in a 14.5% milo-soybean meal basal diet raised the protein equivalent of the diet to 17.3%. The additions of NPN resulted in inferior performance when compared to 1% soybean meal but not when compared to the unsupplemented basal diet. Diammonium citrate (DAC) at 13% of the diet, or urea at 3.4% of the diet, substituted for casein in casein-sucrose rat diets, depressed the ADG of young rats (Fried and DaSilva, 1950). However, when either urea, DAC, or glutamic acid was added to a purified basal diet (essential amino acids as the sole source of N) such that NPN supplied 32.2% of dietary N for protein-depleted adult rats (Frost and Sandy, 1951), ADG was significantly improved. Urea, however, was less effective as a source of non-essential nitrogen than either glutamic acid or DAC. Finlayson and Baumann (1956) fed 5% dietary urea to rats in 20% casein diets. These workers noted a depression in growth of space-fed rats while ad libitum fed rats did not exhibit a depression in growth until urea exceeded 25% of the diet.

The effects of NPN additions to practical and synthetic diets for chickens has been comprehensively reviewed by Featherston (1967).

Effects on NPN on feed conversion

Associated with the effects of inclusion of urea in swine diets on ADG and feed intake, a deleterious effect on FC would be expected as it is a function of these two criteria (Hoefer, 1967). This is generally borne out in the literature with regard to the pig (Braude and Foot, 1942; Hanson and Ferrin, 1955; Hays et al., 1957; Kornegay et al., 1964; Kornegay et al., 1966; Bowland, 1967) and the rat (Frost and Sandy, 1951).

In chicks fed 15 and 17% crude protein diets (Featherston, 1967), FC was generally impaired by NPN (as urea, DAC, or diammonium phosphate) additions, while inclusion of antibiotic tended to overcome depressions in ADG and FC.

The suggestion has been made that the reduction in FC is a result of NH_3 toxicosis, arising from urea hydrolysis (Visek et al., 1959; Visek, 1962; Harbers et al., 1963a), and the subsequent increased energy requirement of the animal for reformation of urea in the liver (Hoefer, 1967). However, studies with rats administered with C^{14}-urea and fed liquid diets deficient in protein (Holtzman and Visek, 1964), indicate that only 1% of the apparent energy spared in antibiotic or resin treated animals was required for conversion of circulating NH_3 to urea. This would, therefore, indicate that differences in FC are not solely due to an increased energy need for urea re-synthesis.

Effects of NPN on blood composition

Increasing blood urea levels (22.2 to 29.3 mg/100 ml) accompanied an increase of dietary urea from 0 to 1.25% of the diet fed to 10.3 kg

crossbred pigs (Hays et al., 1957). Growing pigs fed 17% protein equivalent test diets, containing up to 3.4% dietary urea, were studied with respect to plasma urea and NH_3-N levels by Kornegay et al. (1964). Increasing level of dietary urea, increasing age, and increasing weight were related to increased plasma urea N and decreased plasma NH_3-N levels. Subsequent research (Kornegay et al., 1965b) compared 2.5% urea additions to the basal diet with 2.5% isonitrogenous replacement of protein by urea in the 17% protein basal diet when fed to 44.7 kg pigs. Although hematocrit, hemoglobin, leucocyte, antibody, and serum protein values were unaffected, pigs receiving urea had increased ($P<0.05$) serum urea and reduced serum NH_3 levels 32 days after the start of the trial. Dietary urea, however, did not have any effect on serum urea concentrations at 69 days, implying an adaptive response of the pigs to dietary urea.

Visek (1964) observed that urea moved with relative ease across intestinal membranes; hence, the implication that monogastrics receiving dietary urea would be expected to show elevated blood urea levels in the absence of an efficient urea hydrolysis mechanism. Reduced plasma NH_3 levels in pigs fed diets containing urea (Kornegay et al., 1965b) suggested that the pig may over-compensate for possible increased levels of NH_3 produced in the intestinal tract by bacterial urease. Handford (1961), in studies with dogs, induced NH_3 intoxication through intravenous administration of urease. An elevation ($P<0.01$) of plasma glutamine concentration (8.4 mg/100 ml to 76.3 mg/100 ml, 60 minutes post-administration) prompted the conclusion that glutamine synthesis may serve as a means of NH_3 clearance from the blood when NH_3 is in excess of the amount readily removed by urea formation.

In 10-day-old chicks, urea and DAC as non-essential N sources (in diets containing only the essential amino acids) resulted in increased plasma amino acid concentrations (Featherston, Bird, and Harper, 1962). Similarly, analysis of plasma and muscle tissue from growing rats indicated increased concentration of non-essential amino acids (and their metabolic derivatives) when 8% casein diets were supplemented with glutamic acid, DAC, or their mixture (Swendseid, Hickson, and Freidrich, 1962).

Effects of NPN on nitrogen retention

Limited evidence is available regarding the specific effects of dietary urea on the N retention of monogastric species. Linearly decreasing N retention values were reported (Hays et al., 1957) when 10.3 kg and 32.7 kg pigs were fed up to 1.25% urea in the diet. N retention decreased from 47.1% to 33.9% concomitant with increased blood urea levels (22.2 mg/100 ml rising to 29.3 mg/100 ml) as urea increased from 0 to 1.25% of the diet.

In young adult protein-depleted rats, N retention was improved (Frost and Sandy, 1951) by N supplementation with either glutamic acid, DAC, or urea supplying 32.2% of the N in purified diets containing amino acids as the sole source of N. However, urea was the least effective of these three N sources. Sibbald (1957) reported that the N retention of rats, fed purified amino acid diets containing DAC, was affected more by the ratio of apparent digestible energy to apparent digestible N than by the form of N source. That is, DAC was useful to the rat for maintenance of positive N balance. Similarly, increased N retention by chicks fed NPN has been reported by

Featherston et al. (1962). Ten-day-old chicks fed crystalline essential amino acids as their sole N source exhibited improved N retention when the diets were supplemented isonitrogenously with either urea, DAC, or a mixture of non-essential amino acids.

Studies with humans (Giordano, 1963; Snyderman et al., 1962; Snyderman, 1967) indicated that 2 g of N daily, supplied as urea, ammonium salts, glycine, or glutamic acid to protein-restricted subjects, restored positive N balance, while diets containing only essential amino acid N resulted in a negative N balance.

Fate of N^{15}-labelled compounds in monogastric species

Rats receiving 80 to 170 mg/kg bodyweight of N^{15}-glycine and humans receiving 10 mg/kg bodyweight of N^{15}-glycine in normal diets excreted 30% of the administered N^{15} in 24 hr and 40% (cumulative) in 48 hr post-administration via the urine (Sprinson and Rittenberg, 1949a). N^{15}-urea or N^{15}-ammonium lactate injected into rats was excreted in the urine in the amounts of 83.5% (94.8% as urea) and 55.4% of the injected isotope, respectively, 40 hours after administration (Kornberg and Davies, 1952). Dogs, receiving N^{15}-ammonium citrate, intraperitoneally, excreted 84.4% (as urea) of the administered isotope in the urine during 48 hr following administration (Gaebler et al., 1959).

N^{15} labelling of muscle creatine (Rittenberg, Schoenheimer, and Keston, 1939; Foster, Schoenheimer, and Rittenberg, 1939) and derivation of up to 10% of amide N from administered N^{15}-NPN (Foster et al., 1939) has been reported to occur in rats fed N^{15}-ammonium citrate in casein diets. More recent studies with rats (Kornberg and Davies, 1952; Vitti, Vukmirovich, and Gaebler, 1964) and pigs (Liu et al., 1955) show that

N^{15} of N^{15}-labelled NPN compounds enters the metabolic N pool of these animals as evidenced by N^{15} enrichment of liver amide-N and blood NH_3-N.

Protein fractions precipitated from samples of liver, kidney, intestine, heart, spleen, and skeletal muscle of rats have shown significant enrichment (Rittenberg et al., 1939; Foster et al., 1939; Kornberg and Davies, 1952; Vitti and Gaebler, 1963; Vitti et al., 1964) illustrating that NPN (as N^{15}-ammonium citrate or N^{15}-urea) can serve to some extent as a N source for the synthesis of protein. Similarly, protein from red blood cells and plasma of infant humans exhibited N^{15} enrichment when N was supplied as either N^{15}-urea or N^{15}-ammonium chloride (2 g N equivalent/day). During the administration period, 13 and 4.9% of the N incorporated into plasma and red blood cell protein, respectively, was N^{15} (Snyderman, 1967). Liu et al. (1955) reported that small but significant amounts of urea administered in capsular form were used as a N source by 15.9 kg pigs. N^{15} enrichment of liver and blood protein was approximately 0.02 atom % excess, similar to other results obtained with rats (Bloch, 1946; Kornberg and Davies, 1952). Incorporation of N^{15} was lowest in muscle protein (Liu et al., 1955). Liu et al. (1955) also reported that 0.32% of the administered isotope appearing as non-urea, non-ammonia N in the urine gave conclusive evidence that the administered N^{15}-urea was metabolically active.

To allow examination of the individual amino acids and the degree to which each is enriched, various workers (Rittenberg et al., 1939; Foster et al., 1939; Rose and Dekker, 1956; Vitti et al., 1964) studied hydrolysates of isolated protein fractions from the tissues of test animals fed various forms of N^{15}-NPN. Glutamic acid from liver protein

had the highest isotope concentration (0.046 atom % excess) of four amino acids isolated from liver, intestinal wall, kidney, or muscle of rats receiving 5.8% of total dietary N as N^{15}-ammonium citrate (Rittenberg et al., 1939). Subsequent studies (Foster et al., 1939) confirmed these results. Glutamic and aspartic acids were the most extensively labelled of seven amino acids, though all amino acids studied, with the exception of lysine, showed significant N^{15} enrichment. Rose and Dekker (1956) also reported that glutamic and aspartic acids were more highly enriched (0.962 atom % excess and 0.841 atom % excess, respectively) than tyrosine (0.542 atom % excess), cystine (0.433 atom % excess), proline (0.522 atom % excess), or histidine (0.103 atom % excess) when isolated from protein precipitates of whole rat carcasses. The rats in these experiments were fed two dietary formulations containing isotopic urea (1.23% of diet) furnishing an additional 0.577 g N/100 g of diet. Examination of the heart, liver, spleen, and quadriceps muscle of rats injected with N^{15}-ammonium citrate by various routes (Vitti et al., 1964) showed extensive labelling of glutamic acid and arginine; less extensive, yet significant, labelling was found in seven other isolated amino acids. It is therefore apparent that N of NPN compounds is actively involved in N metabolism (Foster et al., 1939; Rose and Dekker, 1956) as evidenced by the enrichment of isolated amino acids, especially aspartic and glutamic acids.

Ammonia of NPN origin varies in its utility in monogastric metabolism, being influenced by several factors. Foster et al. (1939) reported that a small amount of N^{15}-glycine was isolated from the tissues of a mature rat fed 16% casein and N^{15}-ammonium citrate at a level of

0.6% of the diet. More conclusive evidence of N^{15} incorporation occurred in immature rats, receiving a low protein diet containing 2.25% N as DAC, as indicated by enrichment of six amino acids. Rats given 0.105 meq N^{15} as DAC (Sprinson and Rittenberg, 1949a) or humans receiving 10 mg/kg bodyweight of N^{15}-glycine (Sprinson and Rittenberg, 1949b) excreted nearly all of the administered N^{15} when fed high levels of protein (81% casein for human subjects, 50% casein for rats). Only one-half as much N^{15}, as a percent of that administered, was excreted in the urine when low protein diets were fed. Human subjects receiving a normal level of protein in the diet retained up to 70% of administered N^{15} from N^{15}-glycine in the diet for protein synthesis. Rose and Dekker (1956) reported that 45 g rats, when fed diets supplying essential amino acids as the sole source of N as compared to 18% casein diets, retained 21.7% of administered N^{15} as carcass protein when non-essential amino N was limiting; however, only 3.0% of administered N was retained when all amino acids were furnished as casein. Most recently, it has been illustrated that dogs (Gaebler and Choitz, 1965) and humans (Snyderman, 1967) effectively utilized NPN as N^{15}-ammonium citrate and N^{15}-urea, respectively, when protein intake from normal sources was restricted.

Research has demonstrated that rats (Gaebler et al., 1959; Vitti and Gaebler, 1963; Vitti et al., 1964) and dogs (Gaebler and Choitz, 1965) more effectively utilized the N^{15} of N^{15}-ammonium citrate when treated with growth hormone, as evidenced by urinary excretion data and enrichment of isolated amino acids. Gaebler and Choitz (1965) reported that corticotrophin treatment of dogs receiving N^{15}-ammonium

citrate tended to increase N^{15} excretion via the urine; 93.5% of the administered dose was excreted in hormone-treated dogs while only 84.4% was excreted by the control animals. These workers, however, reported no change in N^{15} retention when α-ketoglutarate, sodium lactate, or pyruvate were supplied as possible NH_3 receptors.

Effects of NPN as the sole source of non-essential nitrogen

Rose, Oesterling, and Womack (1948), in studies with rats, reported improved ADG in those animals receiving nine non-essential amino acids, in addition to the ten essential amino acids, as compared to rats receiving a diet containing only the ten essential amino acids. In later trials, Rose et al. (1949) reported that urea or DAC additions to purified diets containing the ten essential amino acids as the sole N source improved the ADG of rats by 50 and 100%, respectively. These increases in ADG were accompanied by improvements in N retention. It was concluded that the rat required a source of non-essential N and that this requirement was met by NPN compounds such as urea and DAC. Other studies with rats (Lardy and Feldott, 1949, 1950; Rose and Dekker, 1956; Sibbald et al., 1957; Rechcigl, Loosli, and Williams, 1957; Farlin, Hatfield, and Garrigus, 1966; Adkins et al., 1967) confirmed the findings that NPN supplementation of purified diets deficient in non-specific N, but adequate with respect to the essential amino acids, resulted in improved growth.

Featherston et al. (1962), in studies with ten-day-old chicks fed purified diets containing amino acids as the sole source of N, found that urea and DAC substituted isonitrogenously for non-essential amino acids did not reduce ADG and FC. Also, supplementation with urea or

DAC improved N retention in comparison to that of the control diet chicks, which were supplied with minimally required amino acid levels (NRC, 1960) as the sole N source.

Small (Scott, Dean, and Smith, 1963) or significant (Farlin et al., 1968) improvements in ADG of chicks have been reported when urea, DAC, or L-glutamic acid was added to basal purified diets containing only essential amino acid N. Chick diets deficient in non-essential N supplemented with 2.33% biuret caused significant decreases in the ADG and feed intake while supplemental urea or glutamic acid improved ($P < 0.01$) ADG, FC, and feed intake. Chavez, Thomas, and Reid (1966) reported analagous results with laying hens, noting that DAC or diammonium phosphate can effectively supply non-essential N when the diet was adequate with respect to the requirements for essential amino acids. In these studies urea was not utilized by the laying hen as a source of dietary N.

Swendseid, Harris, and Tuttle (1960) reported similar results in young adult humans. Diammonium citrate and glycine in combination were as effective in maintaining N equilibrium as a mixture of non-essential amino acids.

Research with respect to the effects of NPN substitution in purified amino acid diets for swine is more limited. Dudley et al. (1962) found no difference in the utilization of 5 or 10% glutamic acid in diets containing 4.18, 8.36, or 12.54% of amino acid mixtures. Purified amino acid diets, however, failed to support baby pig growth as well as a casein control diet. Mitchell et al. (1964) fed 10 kg pigs glutamic acid as the sole source of non-essential N in diets

containing essential amino acid mixtures (10.7% protein equivalent) and observed that N retention was not dissimilar to that of pigs fed 10% casein diets. Babcock and Markley (1968) reported that weanling pigs, receiving dietary N solely as crystalline amino acids exhibited reduced N retention as compared to pigs fed dietary N as a casein-lactalbumin mixture or as wheat gluten. These authors speculated that this may have been a result of insufficient non-specific N (as glutamine or asparagine) or increased NH_3 requirement, or a combination of both, for the detoxification of HCl of the synthetic amino acids.

Effects of urea supplementation of practical swine diets

Hays et al. (1957) reported that no significant improvement occurred in the ADG or FC of 32.7 kg pigs from the addition of 0.65% urea, 0.04% DL-methionine, 0.25% L-lysine, or a combination of urea and amino acids, to an 8% corn-soybean meal ration. Soldevila and Meade (1964) also observed that no significant interaction of urea and amino acids (lysine and methionine) occurred when added in combination to 13.3% barley rations. Although methionine failed to improve ADG and FC, L-lysine additions tended to improve these criteria. A combination of L-lysine and DL-methionine, however, did not result in further improvements in swine performance. Urea had no effect on performance, either singly or in combination with the other components. When urea replaced corn or soybean meal N (Kornegay, 1966; Kornegay et al., 1966) in the rations fed to 16 to 45 kg pigs, the diets most deficient in lysine, methionine, and cystine resulted in the poorest ADG. Whereas 0.5% L-lysine addition to a diet (in which urea replaced

soybean meal N) tended to depress ADG and feed intake, additions of 0.06% tryptophan and 0.15% DL-methionine tended to abolish the depression in growth. Furthermore, an interaction ($P<0.01$) between lysine and these two amino acids was observed (Kornegay et al., 1966). Bowland (1967) reported, however, that the addition of supplemental lysine (0.19 to 0.57%) to cereal diets for weanling pigs tended to overcome the depression in ADG caused by urea (2 or 3%) substitution for fishmeal N.

Lysine and methionine supplementation of swine diets

Lysine was first shown to be an essential amino acid for swine by Mertz, Shelton, and Beeson (1949). Methionine deficiency was demonstrated later (Shelton, Beeson, and Mertz, 1951; Curtin et al., 1952). Both lysine and methionine tend to be limiting in cereal based diets especially if the diet supplies less than the recommended levels (Subcommittee on Swine Nutrition, National Academy of Sciences--National Research Council, 1968) of total protein. Much of the research in this regard indicates that total protein of the diet can be lowered if amino acid balance is achieved (Evans, 1961, 1962; Bowland, 1962; Nielson et al., 1963; Aldinger and Roberts, 1963).

Additions of L-lysine (0.2 to 0.8% of the diet) to 13.8% protein barley based starter diets (Dinusson, Erickson, and Bolin, 1958) significantly improved the ADG and FC of 11.3 kg pigs. Clawson, Barrick, and Smart (1963), on increasing lysine from 0.26 to 0.66% in the diet, reported a resultant increase in ADG, FC, and feed intake. Similarly, 0.2% L-lysine supplementation of a 13% protein barley diet improved ADG when fed to pigs from 16.5 kg to 50 kg liveweight (Bowland, 1962).

Bell (1964, 1965) also observed an improvement in ADG with the addition of 0.1 and 0.12% L-lysine to 13% protein cereal diets. Other workers, however, have found negative response (Handlin et al., 1962; Schnarre and Tribble, 1962) or no response (Pfander and Tribble, 1955; Sewell and Price, 1962; Davidson, Young, and Thomas, 1962) when swine rations were supplemented with L-lysine.

The protein-sparing effect of lysine in swine diets is evidenced by the comparison of L-lysine supplemented diets low in protein relative to a standard basal. Magruder, Sherman, and Reynolds (1961) observed that performance of pigs provided with a low protein diet and supplemented with 0.1% L-lysine tended to equal that of pigs receiving an unsupplemented high protein diet. Lysine added at a level of 0.2% to a 13% protein barley diet (Bowland, 1962) improved ADG and FC such that these became equivalent to the performance of pigs receiving a practical 16% protein barley-soybean meal diet, when fed from 16.5 to 50 kg liveweight. Other workers have made similar observations when barley diets were fed to growing swine (Bell, 1964, 1965) and when corn-soybean meal diets were fed to finishing swine (Gallo and Pond, 1968).

Methionine has been reported to be the most limiting amino acid in soybean meal (Berry et al., 1962; Kroening, Pond, and Loosli, 1965). Bowland and Asplund (1960) observed that, although lysine additions to a 10% basal wheat diet increased ADG of baby pigs, the addition of DL-methionine or tryptophan to a L-lysine supplemented diet did not result in further improvements. Other workers (Pfander and Tribble, 1955; Sewell and Keen, 1958; Acker, Catron, and Hays, 1959; Chance, Mertz, and Beeson, 1960) reported no improvements when the diet was

supplemented with DL-methionine.

The addition of DL-methionine to soybean meal diets when fed to growing pigs failed to improve N retention (Meade, 1956; Meade et al., 1965; Welch, Cordts, and Van der Noot, 1966). Others (Long et al., 1962; Kroening et al., 1965), however, have reported improved N digestibility and retention when diets of baby or growing pigs were supplemented with DL-methionine.

Evans (1960) reported increased N retention by weanling pigs resulting from the addition of 0.2% L-lysine and 0.1% DL-methionine to 13.8% of protein diets; ADG, FC, and feed intake were similarly improved. Standish and Bowland (1966) reported significant improvements in feed intake, FC, N digestibility, and N retention by baby pigs receiving diets of 12% protein supplemented with L-lysine and DL-methionine.

No response attributable to interaction of L-lysine and DL-methionine when these amino acids were supplemented in growing pig diets was reported (Meade, 1956; Reimer, Meade, and Grant, 1964; Soldevila and Meade, 1964; Meade, Dukelow, and Grant, 1966; Welch et al., 1966); however, contrary to these reports, significant interactive effects of L-lysine and DL-methionine have been observed (Meade et al., 1965; Standish and Bowland, 1966).

Welch et al. (1966) reported that a significant ($P < 0.01$) lysine-tryptophan interaction occurred when these two amino acids were added to the diet of growing barrow pigs, while DL-methionine alone, or in combination with L-lysine, had no effect on performance. Sure (1955) reported that threonine may be more limiting than methionine for pigs

fed low protein barley diets. Studies by Müller and Málek (1967), involving mono-diets based on single cereal grains, also suggested that threonine and tryptophan may be more limiting than methionine in swine diets comprised primarily of cereal grains.

EXPERIMENTAL

Objectives

The general objective of these experiments was to evaluate the use of urea and the amino acids, L-lysine and DL-methionine, as supplements to the diets of swine at two stages of growth.

More specifically, the experiments conducted were designed to study:

1) Effects of 2% dietary urea isonitrogenously replacing fishmeal or soybean meal protein, 0.38% L-lysine and 0.12% DL-methionine supplementation on the ADG, FC, and feed intake of 4-to-11 week old pigs. (Experiment 1, Experiment 2)
2) Effects of L-lysine and DL-methionine supplementation of low protein starter diets, with or without 2% urea, on the ADG, FC, and feed intake of pigs from 4 to 10 weeks of age, in order to provide a negative control comparison. (Experiment 3)
3) Effects of 2% dietary urea, 0.42 or 0.21% L-lysine, and 0.09% DL-methionine on the ADG, FC, feed intake, and carcass quality of swine grown to market weight. (Experiment 4)
4) Nitrogen and energy metabolism of castrate male and female pigs fed supplemental urea alone or with L-lysine, DL-methionine, or both at 25 kg and 65 kg body weight. (Experiment 5)
5) The fate of N^{15}, supplied as N^{15}-urea in the diet of 10 kg barrows, in terms of excretion and incorporation into body tissue protein. (Experiment 7). A preliminary trial was required to determine the effects of adding 3% dietary urea to a standard 22% protein diet on the rate of N excretion. (Experiment 6)

All growth experiments were factorially designed in a 2 x 3 x 2 arrangement, factors including sex, three amino acid combinations (no amino acid additions, lysine, or lysine plus methionine), and 0 and 2% urea with the exception of Experiment 3 (negative control trial) which was designed as a 2 x 7 factorial, with sex and seven ration treatments as factors.

The metabolism experiment (Experiment 5) was designed as a 2 x 3 x 2 x 2 factorial, in two replicates, factors including sex, supplemental amino acids, urea, and body weight, respectively.

A complete block design was utilized in the study of N^{15}-urea utilization, involving two replicates and two treatments (1.25 g N^{15}-urea without supplemental amino acids and 1.25 g N^{15}-urea plus supplemental L-lysine and DL-methionine in the diet).

Formulation of Experimental Diets

The formulation and composition of the experimental diets is shown in Table 1.

Starter period diets

A standard practical starter diet (Bowland, 1965) served as the basal, having been formulated by electronic computation to meet the requirements of 10 to 20 kg pigs as defined by the Sub-committee on Swine Nutrition of the National Academy of Sciences--National Research Council (1964).

Urea[1] was isonitrogenously substituted for fishmeal. Sucrose was added to adjust the weight when urea replaced fishmeal N.

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[1] Sherritt Gordon Mines, Fort Saskatchewan, Alberta; 45% N, prilled feed compound.

Table I. Formulation and composition of experimental diets

Stage of Growth	Starter Period (4 to 11 weeks of age)									Grower-Finisher Period (11 weeks to market)					
Diet Number	1, 1A	2A	2, 3A	3	4A	4, 5A	5	6A	6, 7A	1 B	2 B	3 B	4 B	5 B	6 B
Fed in Experiments	1,2,3	3	1,2,3, 6,7	1,2	3	1,2,3	1,2	3	1,2,3, 6,7	4	4	4	4	4	4
Ingredients															
Ground wheat	61.3	62.64	60.64	61.3	62.64	60.64	61.3	62.64	60.64	7.0	7.0	7.0	7.0	7.0	7.0
Ground oats	---	---	---	---	---	---	---	---	---	20.0	20.0	20.0	20.0	20.0	20.0
Ground barley	---	---	---	---	---	---	---	---	---	50.95	60.07	50.95	60.07	50.95	60.07
Oat groats	4.1	4.1	4.1	4.1	4.1	4.1	4.1	4.1	4.1	0.50	0.50	0.50	0.50	0.50	0.50
Stabilized tallow	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	4.75	6.75	4.75	6.75	4.75	6.75
Sucrose	5.0	8.5	8.5	5.0	8.5	8.5	5.0	8.5	8.5	---	---	---	---	---	---
Dried molasses	2.4	2.4	2.4	2.4	2.4	2.4	2.4	2.4	2.4	---	---	---	---	---	---
Dry skimmilk powder	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	---	---	---	---	---	---
Fishmeal (72%)	10.0	4.5	4.5	10.0	4.5	4.5	10.0	4.5	4.5	1.50	0.23	1.50	0.23	1.50	0.23
Meat meal (55%)	1.2	1.2	1.2	1.2	1.2	1.2	1.2	1.2	1.2	0.50	0.50	0.50	0.50	0.50	0.50
Soybean meal (44%)	6.0	6.0	6.0	5.62	5.62	5.62	5.5	5.5	5.5	12.80	0.53	12.59	0.11	12.50	0.02
Urea (45% N)	---	---	2.0	---	---	2.0	---	---	2.0	---	2.00	---	2.00	---	2.00
Ground limestone	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	1.00	1.00	1.00	1.00	1.00	1.00
Dicalcium phosphate	0.4	1.0	1.0	0.2	1.0	1.0	0.2	1.0	1.0	---	0.42	---	0.42	---	0.42
Iodized salt	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.40	0.40	0.40	0.40	0.40	0.40
Trace mineral mix [1]	0.15	0.21	0.21	0.15	0.21	0.21	0.15	0.21	0.21	0.15	0.15	0.15	0.15	0.15	0.15
Zinc sulfate	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
Antibiotic supplement [2]	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.10	0.10	0.10	0.10	0.10	0.10
Vitamin B complex mix [3]	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20
Vitamin B_{12} (9 mg/kg)	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10
Vitamins A + D_2 [4]	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
L-lysine HCl	0	0	0	0.38	0.38	0.38	0.38	0.38	0.38	---	---	0.21	0.42	0.21	0.42
DL-methionine (90%)	0	0	0	0	0	0	0.12	0.12	0.12	---	---	---	---	0.09	0.09
Total	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
Composition															
(N x 6.25) (Anal.) %	21.0	18.13	23.0	20.2	18.19	22.5	20.7	18.81	22.7	15.3	15.5	14.9	15.2	15.0	15.3
Gross energy (Anal.)kcal/kg	4188	4081	4098	4317	4049	4213	4123	4150	4050	4234	4463	4345	4235	4301	4238
Lysine (Calc.)	1.35	0.96	0.95	1.70	1.32	1.32	1.70	1.32	1.31	0.83	0.43	1.04	0.84	1.04	0.83
Methionine+cystine (Calc.) %	0.74	0.54	0.54	0.74	0.54	0.54	0.86	0.66	0.66	0.48	0.39	0.47	0.39	0.56	0.48

1 Contains the following per kg of mix: cobalt carbonate, 2.28 g; copper sulfate ($CuSO_4 \cdot 5H_2O$), 24.50 g; ethylene diamine dihydroiodide, 1.30 g; ferrous carbonate, 234.80 g; manganous oxide, 47.73 g; zinc oxide, 2.96 g; ground limestone, 686.43 g.

2 Aurofac 10 - 4.54 gm chlorotetracycline/kg, Cyaninid of Canada Ltd.

3 Contains the following per kg of vitamin mix: riboflavin, 4.4 g; calcium pantothenate, 8.8 g; niacin, 19.8 g; choline chloride, 21.45 g; folic acid, 132.0 mg.

4 To supply 440,000 i.u. vitamin A and 44,000 i.u. vitamin D_2 per 100 kg of rations 1 to 6, 1A to 7A; 220,000 i.u. vitamin A and 44,000 i.u. vit D_2 per 100 kg of rations 1B to 6B.

L-lysine monohydrochloride[1] and DL-methionine[2] were added at the expense of soybean meal in the amounts by which these were reduced when urea and sucrose were subsituted for fishmeal.

Removal of fishmeal N and subsequent replacement with urea N necessitated upgrading of the level of calcium and phosphorous supplied. This was accomplished by increasing the level of dicalcium phosphate. Trace mineral levels were similarly increased.

In order to evaluate the effects of restricted protein (18.1%) in the diet, without urea or amino acid supplementation, a negative control diet was formulated (Experiment 3). Urea and amino acids were manipulated as previously discussed such that comparison of amino acid supplementation of both urea- and non-urea-containing, low-protein equivalent diets could be made.

Grower-finisher period diets

Substitution of urea for fishmeal and soybean meal N was made on the same basis as in the case of starter period diets except that weight adjustment was made through the addition of barley when urea replaced fishmeal or soybean meal N.

Crude protein equivalent (% N x 6.25) and digestible energy content were held constant in all diet computations. Also, all diets were computed to meet the requirements of 35 to 60 kg pigs as defined by the Sub-committee on Swine Nutrition of the National Academy of Sciences--National Research Council (1968).

L-lysine and DL-methionine additions were made at the expense of soybean meal, except in the cases of Diets 3B and 5B wherein only half

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1 Chas. Pfizer Co., Ltd., Montreal, P. Q.; Feed grade, 97% L-lysine.

2 Philipp Brothers (Canada) Ltd., 1440 St. Catherine Street West, Montreal 25, P.Q.; Feed grade, 98% DL-methionine.

of the level of reduction was added.

All diets were mixed and bagged at the University of Alberta elevator and stored in the barns of their respective use.

Methods and Procedures

Feeding trials were conducted from February 1, 1968 to September 4, 1968 and from January 8, 1969 to February 19, 1969. Metabolism trials were conducted with pigs at 25 kg bodyweight and at 65 kg bodyweight between the dates of April 22 to May 23, 1968 and June 14 to October 3, 1968, respectively.

The pigs used in these experiments were of Lacombe-Yorkshire or purebred Yorkshire litters. All were managed according to the normal routine followed at the University of Alberta Livestock Farm, including:

1) Identification by ear-notching and clipping of black teeth at 8 hr after birth.
2) Injection at 4 to 5 days of age with 2 ml Imposil 200[1] (100 mg iron/ml) for prevention of anemia.
3) Castration of males at 10 days of age.
4) Weaning at 3 weeks of age.
5) Erysipelas bacterin[2] injection, Lindane treatment for control of external parasites, and treatment for ascarid control[3] at 6 to 8 weeks of age. Lindane treatment was repeated 5 to 6 days after the initial treatment.

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[1] Fisons (Canada) Limited, 26 Andrew Place, Don Mills, Ont.

[2] Chas. Pfizer and Co., Ltd., Agricultural Division, New York, New York.

[3] Dowzene DHC, product of Dow Chemical of Canada Ltd., Toronto, Ont.

Pigs in each feeding experiment were weighed and randomly allotted to the respective diets as they reached approximately four weeks of age. They were balanced with respect to sex where possible. Unequal numbers of barrows and gilts were used in Experiments 2 and 4 in which four gilts and two barrows were included in each treatment group.

In the metabolism experiments, pigs were allotted as they reached 25 kg and 65 kg liveweight, respectively. The same pigs were used in both collection periods to provide a comparison of the effects of age on N and energy metabolism.

Experiment 1 -- Average daily gain and feed conversion of pigs 4 to 10 weeks of age

Forty-eight Yorkshire and Lacombe-Yorkshire weanling pigs were allotted, at an average age of 26 days, in the Muttart feeding barn at the University Livestock Farm. Diets 1 to 6 (Table 1) were provided.

The pigs were allowed access to feed for three one-hour intervals daily (7 AM, 11:30 AM, and 4:30 PM). When not feeding, the pigs were allowed to run in groups of four. Water was provided by means of automatic bowls in the unrestricted pen area.

Individual feed consumption and bodyweights were recorded weekly, feed weigh-backs including feed unconsumed at the end of each week. Weight data were recorded similarly in Experiments 2 and 3.

Experiment 2 -- Average daily gain and feed conversion of pigs 4 to 11 weeks of age

Thirty-six Yorkshire and Lacombe-Yorkshire weanling pigs were randomly allotted to individual feeding pens (dimensions 61 cm x 122 cm) with half-slotted floors in the new feeding barn. The pigs had access

to feed at all times. Water was supplied to each pen by automatic watering cups.

Experiment 3 -- Average daily gain and feed conversion of 4 to 10 week old pigs fed low protein diets

Twenty-eight Yorkshire and Lacombe-Yorkshire weanling pigs were allotted to individual feeding pens in the new barn[1]. Diets 1A to 7A were provided to seven groups of pigs, each group balanced according to sex.

Experiment 4 -- Average daily gain, feed conversion, and carcass quality of pigs raised to market weight on urea-containing diets

When the pigs from Experiment 2 reached 11 weeks of age, they were fed diets 1B to 6B. At 15 weeks of age, they were moved to the L-barn finisher section and penned according to dietary treatment in six groups of four gilts and six groups of two barrows. Bodyweight gain and feed consumption of each group was recorded weekly. As they reached 88 kg liveweight, the pigs were marketed and ROP carcass data[2] were obtained.

Experiment 5 -- Nitrogen and energy metabolism of urea-fed pigs at two stages of growth

As they reached 25 kg liveweight and 65 kg liveweight, two groups of six gilts and two groups of six barrows, representing each dietary treatment, were confined in metabolism cages, as described by Castell (1967).

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1 Muttart feeding barn and new feeding barn are the common names applied to barns at the University of Alberta Livestock Farm and are therefore used for identification in this thesis.

2 Anonymous; Animal Industry Division, Canada Department of Agriculture.

Following a three-day period during which the pigs became accustomed to the cages, total feces and urine were collected for three days. A daily feed allowance, based on the voluntary average daily consumption during the previous week, was provided in one portion.

The cages were cleaned prior to starting collections. Urine was collected continuously in plastic containers to which had been added 25 ml of 25% (V/V) H_2SO_4. Feces were collected and weighed twice daily and stored in plastic bags in a refrigerator.

Total urine was measured and a 250 ml aliquot was taken and stored in a sealed glass jar at 3° C until analyzed for N content. A 200 ml aliquot of each urine sample was freeze-dried and retained for gross energy determinations. Duplicate 5 ml samples were freeze-dried simultaneously for urine dry matter computation.

Total feces of each pig were pooled, mixed thoroughly, and a 50 g aliquot taken for fecal N analysis. This sample was wrapped in plastic and stored in a sealed jar at 3° C until analysis. The remaining feces were dried at 60° C for 48 hr in a forced air oven[1], allowed to equilibrate with atmospheric moisture for 48 hr, and weighed. Dried fecal samples were ground in a laboratory grinder[2] and saved for gross energy determinations.

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1 Style V31, Despatch Oven Co., Minneapolis, Minnesota, U.S.A.

2 C & N Laboratory Mill, Size 8, 2mm mesh screen; Christy and Norris Ltd., Chelmsford, England.

Experiment 6 -- Preliminary study of the nitrogen excretion pattern of 22 kg pigs fed a high level of urea

In order to estimate the period of maximum nitrogen availability following urea ingestion, it was decided first to determine the rate and pattern of excretion of N.

Three female pigs, with an average weight of 22.7 kg, were confined to metabolism cages and fed 950 g of basal Diet 1 in daily single feedings. Since a feed allowance of 950 g approximated 80% of the voluntary intake, complete ingestion of each feeding was ensured. A three-day period to allow the pigs to become accustomed to the crates was allowed, similar to earlier metabolism trials.

A single feeding consisting of 97% basal Diet 1 and 3% urea (total 950 g) was provided. Urine was collected for a 48 hr period. Basal Diet 1 was provided in subsequent feedings.

Urine was collected in beakers containing 10 ml of 25% (V/V) H_2SO_4. Precautions were taken to prevent fecal contamination of the urine. Volume of each urination was measured and the time of urination recorded. Each quantity of urine was saved in individual sealed glass jars, labelled, and stored in a refrigerator at 3^{o} C for subsequent N analysis.

Experiment 7 -- Utilization of N^{15}-urea as a source of nitrogen for protein synthesis

Administration of N^{15}-urea

Four male weanling pigs, average weight of 7.3 kg, were allotted to Diets 2 and 6 and housed in two feeding pens in the new barn for 14 days to become accustomed to urea in the diet.

At an average weight of 10.3 kg the pigs were confined in the

metabolism cages and fed the same diets as they received during the initial period. A single daily feeding of the amount approximating the voluntary intake, based on feed consumption during the previous week, was implemented to ensure that all the offered feed was eaten.

Following a three-day period after introduction to the metabolism cages, 150 g of Diets 2 and 6, containing 1.25 g N^{15}-urea[1] (97.9 atom % N^{15}), was supplied in three equal portions to ensure that all of the N^{15}-urea was consumed by each pig. For this feeding, 150 g of each of Diets 2 and 6 were hand mixed so that 1.25 g of N^{15}-urea replaced the normal 2% level (3.02 g urea/150 g of diet) of non-labelled urea. The remaining 1.77 g of urea were added to 303 g each of Diets 2 and 6 and fed to the pigs after all the feed containing N^{15}-urea had been consumed.

Sample collection

Feces and urine were collected for a 96 hr period following the feeding of N^{15}-urea. Feces were collected, commencing with the feeding of the N^{15}-urea, three times daily. Each collection was weighed, homogenized with approximately equal volumes of 10% (V/V) H_2SO_4 in a Waring blender[2], sealed in a glass container, and stored in a refrigerator for subsequent analysis.

Urine was collected in plastic containers with 20 ml of 25% (V/V) H_2SO_4. Urine volume was measured three times daily, total urine saved in glass jars and stored in a refrigerator at 3° C. Urine samples were

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1 Bio-Rad Laboratories, 32nd & Griffin, Richmond, California, U.S.A.

2 Model 1042, Waring Products Company, Div. of Dynamics Corp. of America; Winsted, Connecticut, U.S.A.

pooled into two batches of 0 to 48 hr and 48 to 96 hr following N^{15}-urea ingestion.

At 96 hr post-administration, a 40 ml blood sample was withdrawn by anterior vena cava puncture (Carle and Dewhirst, 1942) into plastic centrifuge tubes containing a drop of heparin. The blood samples were immediately cooled to 3° C in a refrigerator. The pigs were then killed by exsanguination and tissue samples were removed immediately.

The entire liver was removed, washed free of residual blood in physiological saline solution (0.9 g NaCl per 100 ml), weighed, cut into 1.3 sq cm cubes, and frozen in dry ice. A section of the longissimus dorsi and cross-section of the gluteus maximus muscles were taken and treated in the same manner as the liver.

The intestinal tract was removed, emptied of contents, and washed with distilled water. Scrapings of the intestinal mucosa were taken from the anterior one meter of the intestine. The intestinal scrapings were packaged, weighed, and frozen in dry ice.

Blood samples were centrifuged for twenty minutes at 5,000 rpm to separate the cells from the plasma. The plasma was decanted, transferred to clean plastic bottles, and frozen. Blood cells were washed in equal volumes of heparinized saline, centrifuged, and the supernatant was discarded. The precipitated cells were sealed and stored in plastic centrifuge tubes at -30° C. Tissue samples and blood samples were kept frozen in storage at -30° C until required for analysis.

Preparation of samples

Entire liver, longissimus dorsi, and gluteus maximus muscle samples were thawed and homogenized individually with physiological saline in a

Waring blender. The total volume of homogenate was measured in a graduated cylinder. Intestinal scrapings were homogenized in a Virtis blender[1] and the volume of homogenate measured. In all cases, two to five drops of capryl alcohol were added as an antifoaming agent.

Immediately following homogenization, an aliquot of the homogenate was taken by pipette and transferred to plastic centrifuge tubes to which an equal volume of 20% (W/V) trichloroacetic acid was added. Following centrifugation (12,000 rpm for 30 minutes), the supernatant was discarded and the precipitate resuspended in 25 ml of 20% trichloroacetic acid. The suspension was heated in a boiling water bath for 30 minutes to dissolve nucleic acids and then centrifuged. The supernatant was discarded and the entire precipitate transferred to a Kjeldahl flask for digestion.

Plasma and blood cell fractions were treated with equal volumes of 20% trichloroacetic acid, centrifuged, and washed with hot trichloroacetic acid as described above. The precipitate was then resuspended in 30 ml of 95% ethanol to remove residual trichloroacetic acid and centrifuged. The precipitate was resuspended in anhydrous ether and centrifuged. It was then dried in a forced air oven at 75° C for 24 hr. Weighed quantities of the powdered material thus obtained were transferred to Kjeldahl flasks for digestion.

Methods of chemical analyses

Feeding and metabolism trials

Gross N values of feed, feces, and urine were determined by the Kjeldahl method (AOAC, 1960). Fecal N was determined in 2.0 g moist

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[1] Virtis Company; Yonkers, New York, U.S.A.

feces samples; urine N was determined in 10 ml aliquots of urine. Catalyst used in the digestion was a commercial Kel-Pak[1]; NH_3 was distilled into 50 ml of 4% boric acid.

Feces and urine samples were prepared for gross energy analysis on a Parr Oxygen Bomb Calorimeter[2] in the following manner. Feces were dried at 60° C for 48 hr in a forced air oven and allowed to equilibrate with atmospheric moisture for 48 hr. They were then ground in a laboratory mill to pass through a 2 mm mesh screen. Urine samples of approximately 200 ml were freeze-dried. Approximately 1 g of urine dry matter was used for gross energy analysis. Air dry feed samples were analyzed for gross energy without additional preparation. All analyses were done in duplicate, except in the case of feed samples which were analyzed in triplicate.

N^{15}-urea experiment

Samples for N^{15} analysis by mass spectrometer were digested by a modified macro-Kjeldahl procedure. Catalyst used was 10 ml of mercuric sulfate solution (Hiller, Plazin, and Van Slyke, 1948) to ensure complete liberation of the N from less readily degradable compounds such as lysine (Sprinson and Rittenberg, 1948). Use of mercuric sulfate rather than copper sulfate as catalyst, furthermore, prevented contamination of the final ammonium sulfate solution by cupric ions that may be carried over during distillation. Cupric ions

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1 Supplies K_2SO_4, $CuSO_4$, and HgO. Matheson Scientific, East Rutherford, New Jersey, U.S.A.

2 Parr Instrument Company, Moline, Illinois, U.S.A.

tend to decompose the hypobromite solution, used in N liberation (Sprinson and Rittenberg, 1949a), by accelerating the evolution of oxygen.

Samples were digested for 12 hr. Although shorter than the digestion period used by Sprinson and Rittenberg (1949a) a 12 hr digestion period was considered adequate in view of the findings of Sprinson and Rittenberg (1948) and Hiller et al. (1948). During the extended digestion, concentrated H_2SO_4 was added in 10 ml quantities as required (Taras, 1958).

Ammonia was distilled into 50 ml of 0.1634 N H_2SO_4 in a 250 ml volumetric flask. A regular macro-Kjeldahl distillation unit was used. Prior to each distillation, each unit was cleaned by distilling 300 ml of demineralized water through it. Zinc dust (2.0 g), as recommended by Hiller et al. (1948), 300 ml of demineralized water, and 110 ml of 40% NaOH were added to each flask for the liberation of NH_3. Approximately 150 ml of distillate were collected.

The volumetric flasks were removed and made up to volume. Two 50 ml aliquots of distillate were pipetted into individual 200 ml Erlenmeyer flasks and titrated with 0.0995 N NaOH (CO_2 free) to determine excess acid as a means of determining total N. Tashiro's indicator as described by Conway and Byrne (1933) was used. The remaining 150 ml of distillate were transferred to clean plastic bottles and frozen at -30° C until required for N_2 liberation.

Liberation of N_2 gas was by the method of Sprinson and Rittenberg (1949a) using a modified high vacuum apparatus equipped with a mercury diffusion pump. A liquid N_2 cold trap was used to collect

condensable impurities.

Thirty to 50 ml of each sample, depending on the NH_3 content, were evaporated to a total volume of 1 to 1.5 ml. Hypobromite (NaOBr) reagent was diluted by one-third volume of demineralized water prior to use. To prevent oxygen liberation from the hypobromite, 0.1% of potassium iodide (KI) was added to the solution (Sims and Cocking, 1958).

After the break-seal tubes had been welded on, the system was tested for air leaks by evacuating to approximately 10^{-6} mm (McLeod gauge) of pressure. Once a suitable vacuum was achieved, the Y-tubes were detached. To one arm was introduced 1 to 1.5 ml of hypobromite solution, sufficient to oxidize 10 to 12 mg of NH_3 (Sprinson and Rittenberg, 1949a). An equal volume of ammonium sulfate concentrate was transferred to the other arm of the Y-tube. The Y-tubes were reattached and partially evacuated, after which tubes and contents were quickly frozen in liquid N. The system and Y-tubes were evacuated once again to 10^{-6} mm.

Once the desired pressure was achieved, the Y-tubes were closed off from the system, removed, and the hypobromite mixed with the sample. The Y-tubes were then reattached, frozen, and kept closed. A third vacuum was drawn to evacuate the system and the attached break-seal tubes.

On obtaining a vacuum of 10^{-6} mm of pressure, the break-seal tubes were closed off from the system and opened to the Y-tubes allowing the entry of liberated N_2 gas. With the system held under vacuum, the break-seal tubes were removed by heating. The contents of the break-

seal tubes were analyzed in a low resolution cycloidal focussing mass spectrometer.[1]

Samples to which N_2 of the air contributed more than 5%, as determined by the height of the oxygen-32 peak relative to the nitrogen-28 peak, were discarded and repeat analyses carried out (Sprinson and Rittenberg, 1949a).

Methods of statistical analyses

Analysis of variance was employed in all determinations of statistical significance. Bodyweight gain and feed consumption data of the pigs in Experiment 1 were transformed to metric units by division of the number of pounds by 2.2. To transform the mean squares to the units of the mean, each was divided by a factor of 4.84. Similarly, means of carcass measurements were transformed from inches or square inches to centimeters by multiplying by 2.54 or 6.54, respectively, and the mean squares in the analyses of variance were adjusted accordingly.

Analysis of variance was conducted using both a 2 x 3 x 2 factorial design and a 2 x 6 factorial design in Experiment 1 and 2. Use of the 2 x 6 design facilitated comparison of group means with those of Experiment 3. Also, group means were examined in addition to factor means to allow comparison of the individual dietary treatments.

In those experiments containing equal numbers of barrows and gilts (Experiments 1, 3, and 5), analyses of variance were conducted, using the appropriate experimental design, on the raw data. Due to unequal numbers of each sex in Experiments 2 and 4, analysis of variance

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[1] Department of Chemistry, University of Alberta, Edmonton, Alberta.

for disproportionate sub-class numbers was conducted according to the method described by Snedecor (1956). In accordance with this procedure, a preliminary analysis of variance was conducted using the mean of each group. The "individual error mean square", calculated from the within-treatment variation and adjusted to a mean basis by multiplication by the reciprocal of the harmonic mean of the number of animals in each group, was used in testing for significance. An exception to this procedure was in the case of Experiment 5 growth data. Since bodyweight gain and feed consumption data were recorded on a group basis, only the means of each group were subjected to analysis of variance.

All analyses of variance were conducted via a remote terminal APL/360 computer system using the Complete Factorial ANOVA program (Smillie, 1969). Analysis of variance of the data in Experiment 7 was done using the One-Way ANOVA2 (Smillie, 1969).

Four pigs died on test in Experiment 5; however, growth data were available for one of these since death occurred on the day the pig was to be marketed. Missing values for the remaining three were not calculated since group bodyweight gain and feed consumption were calculated on the basis of the total number of pig days per group.[1]

Missing carcass data were similarly ignored except in the case of loss of data of both males from dietary treatment 4B. In this case, the method of Steel and Torrie (1960) was used for calculation of the missing mean. Consequently, one degree of freedom was dropped from the error mean square.

Duncan's new multiple range test (Steel and Torrie, 1960) was

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[1] R. T. Hardin, Personal communication.

used to test for significant differences between three or more means. When comparisons were made between treatment means, as opposed to factor means, the standard error of the mean was derived using the pooled degrees of freedom of the appropriate main effects and interactions.

RESULTS AND DISCUSSION

Experiments 1, 2, and 3 -- Response of starting pigs to urea, L-lysine, and DL-methionine--Daily gain, daily feed, and feed conversion

Factor means of Experiments 1 and 2 are presented in Table 2. Corresponding mean square values are shown in Appendix Table (i).

Dietary treatment means of Experiments 1 and 2 are shown in Table 3, and those of Experiment 3 are shown in Table 4. Mean squares of analysis of variance of the 2 x 6 factorial design are presented in Appendix Tables (ii) and (iii).

Experiment 1

Urea reduced ($P<0.01$) ADG, daily feed consumption, and resulted in poorer ($P<0.01$) FC. This is in agreement with results of Hays et al. (1957) who noted deleterious effects of urea in excess of 20% protein equivalent in the diets of 10.3 kg pigs. Similar reductions in ADG, feed consumption, and FC were reported by Bowland (1967) who tested similar diets containing urea.

Amino acid supplementation improved ($P<0.01$) FC; it did not, however, significantly influence ADG or feed consumption. Comparison of treatment means indicates that the depressions in ADG, feed intake, and FC caused by urea were overcome by additions of lysine. No additional improvements resulted from the supplementation of the diets with DL-methionine in combination with L-lysine.

Additions of L-lysine to the basal diet did not result in significant improvements. This agrees with the results of Bowland (1967) who

Table 2. Experiments 1 and 2 -- Factor means of daily feed consumption, daily gain, and feed conversion

Factor	Experiment 1 (4 to 10 weeks)				Experiment 2 (4 to 11 weeks)			
	No. of Pigs	Daily Gain kg	Daily Feed kg	Feed Conversion[1] kg/kg gain	No. of pigs	Daily Gain kg	Daily Feed kg	Feed Conversion kg/kg gain
Urea (%)		**	**	**				
0	24	0.40	0.73	1.82	18	0.52	1.08	2.08
2	24	0.32	0.64	2.02	18	0.51	1.12	2.20
Amino acids								
0	16	0.34	0.67	2.03[a]	12	0.50	1.02	2.08
L-lysine	16	0.37	0.69	1.88[b]	12	0.51	1.09	2.13
L-lysine and DL-methionine	16	0.37	0.69	1.88[b]	12	0.54	1.18	2.21
Sex								
Barrows	24	0.37	0.70	1.90	12	0.52	1.15	2.21
Gilts	24	0.35	0.66	1.93	24	0.51	1.04	2.07

[1] Means sharing a common superscript are not significantly ($P < 0.05$) different from each other.

** Following means are significantly ($P < 0.01$) different.

Table 3. Experiments 1 and 2 -- Treatment group means of daily feed consumption, daily gain, and feed conversion

	Diet Number					
	1	2	3	4	5	6
N x 6.25 (%)	21.0	23.0	20.2	22.5	20.7	22.7
Urea (%)	0	2	0	2	0	2
L-lysine (%)	0	0	0.38	0.38	0.38	0.38
Dl-methionine (%)	0	0	0	0	0.12	0.12
Experiment 1[1]						
Daily gain (kg)	0.39[ab]	0.28[c]	0.39[ab]	0.34[b]	0.41[a]	0.34[b]
Daily feed (kg)	0.72	0.61	0.72	0.66	0.74	0.64
Feed conversion[2]	1.84[a]	2.22[b]	1.83[a]	1.92[a]	1.78[a]	1.91[a]
Experiment 2						
Daily gain (kg)	0.52	0.48	0.50	0.53	0.55	0.52
Daily feed (kg)	1.02	1.04	1.00	1.17	1.22	1.14
Feed conversion[2]	1.98	2.17	2.00	2.21	2.22	2.19

[1] Means sharing a common superscript are not significantly ($P < 0.05$) different from each other.

[2] Kg of feed consumed per kg of bodyweight gain.

noted that the basal diet used, which was the same formulation as Diet 1, appeared to be adequate with respect to lysine.

Sex was not a significant factor on the performance of the pigs in Experiment 1, agreeing, thereby, with the results of Anderson and Bowland (1967) who reported that sex did not have an influence on ADG or FC of 6.0 kg pigs.

In general, the results of Experiment 1 do not indicate that the N of urea was used to any extent by the pigs. Moreover, its inclusion in the diet at a level of 2% contributed to depressions ($P<0.01$) in ADG and poorer FC. Amino acid additions in the form of lysine, or a combination of L-lysine and DL-methionine, tended to mask the effects of urea additions to the diet. Lysine added at a level of 0.38% gave as good FC as additions of L-lysine plus DL-methionine when supplemented to urea diets. Addition of the amino acids to the basal diet did not improve response of the pigs, indicating adequacy with respect to these nutrients.

Experiment 2

Substitution of 2% urea for fishmeal N did not significantly affect ADG, feed consumption, or FC of the pigs in Experiment 2, unlike the results of Experiment 1. However, comparison of treatment means (Table 3) of the two experiments indicate similar trends in ADG and FC resulting from urea addition to the diets; feed intake was not similarly affected. Feed intake in Experiment 2 tended to increase when urea and L-lysine were added in combination while an opposing trend was evident in Experiment 1.

Additions of L-lysine, or L-lysine plus DL-methionine, to the diet,

either with or without 2% urea, resulted in non-significant increases in feed consumption (Table 2). Similar results have been reported by Anderson and Bowland (1967) who observed increased feed consumption resulting from L-lysine supplementation of the diets of young pigs. In general, L-lysine or combined L-lysine and DL-methionine additions tended to influence performance in a similar manner to that in Experiment 1.

Hays et al. (1957) found no improvements in ADG or FC on addition of 0.04% DL-methionine and 0.25% L-lysine to corn-soybean meal diets containing 0.65% urea. Soldevila and Meade (1964) similarly did not observe any improvements in performance resulting from additions of L-lysine and DL-methionine to 13.3% protein barley diets containing urea.

As in Experiment 1, sex did not have any significant influence on performance.

The ADG of the pigs in Experiment 2 was 0.52 kg/day while the ADG of those in Experiment 1 was 0.39 kg/day; feed consumption was also greater in Experiment 2 than in Experiment 1 (1.03 kg/day versus 0.69 kg/day, respectively). The disparity in performance of the pigs in the two experiments may have been the result of different methods of feeding.

The pigs in Experiment 1 were limit fed while those in Experiment 2 were fed ad libitum. Urea reduced ($P < 0.01$) ADG and daily feed, and resulted in poorer ($P < 0.01$) FC in Experiment 1 but not in Experiment 2, suggesting that a relationship exists between inclusion of urea in the diet and the method of feeding. Finlayson and Baumann (1956)

reported that growth of space-fed rats was depressed when 5% urea was added to 20% casein diets; however, rats fed ad libitum did not show similar depressions in growth until urea comprised 25% of the diet. This would suggest a tendency of limit-fed or space-fed animals to ingest large amounts of urea during a short time period, hence resulting in rapid degradation of the urea and poor utilization or increased toxicity relative to those fed ad libitum.

The effects of urea and L-lysine, or L-lysine in combination with DL-methionine, in Experiment 2 were similar to the results obtained in Experiment 1. Differences in response of the pigs to amino acid supplementation in the two experiments appear to have resulted from the respective feeding systems employed (i.e. limit versus self). No positive evidence of urea utilization or relationships between L-lysine, DL-methionine, and urea was obtained, presumably associated with high protein content of the diets.

Experiment 3

Since the effects of amino acid additions exclusive of 2% urea additions were not discernible from the results of Experiments 1 and 2, a low protein negative control diet (2A) was formulated to provide this comparison.

Diets 1A, 3A, 5A, and 7A, which were identical to Diets 1, 2, 4, and 6 of Experiments 1 and 2, supported ADG, feed intake, and FC closely duplicating that of Experiment 2. These data support the suggestion that ad libitum consumption of urea-containing diets lessened the depressant effects of urea.

Reduction of protein from 21.6 to 17.9% did not significantly affect

Table 4. Experiment 3 -- Means of daily feed consumption, daily gain, and feed conversion

	Diet Number						
	1A	2A	3A	4A	5A	6A	7A
N x 6.25 (%)	21.6	17.9	23.2	17.7	23.0	17.6	23.0
Urea (%)	0	0	2	0	2	0	2
L-lysine (%)	0	0	0	0.38	0.38	0.38	0.38
DL-methionine (%)	0	0	0	0	0	0.12	0.12
Barrows							
Daily gain (kg)	0.54	0.53	0.50	0.56	0.50	0.60	0.55
Daily feed (kg)	1.51	1.32	1.15	1.17	1.02	1.18	1.14
Feed conversion[1]	2.74	2.50	2.27	2.07	2.02	1.99	2.04
Gilts							
Daily gain (kg)	0.56	0.46	0.50	0.54	0.55	0.49	0.52
Daily feed (kg)	1.22	1.08	1.14	1.10	1.26	1.00	1.21
Feed conversion[1]	2.13	2.32	2.30	2.00	2.27	2.03	2.28
Average							
Daily gain (kg)	0.55	0.49	0.50	0.55	0.52	0.54	0.54
Daily feed (kg)	1.36	1.20	1.14	1.14	1.14	1.09	1.18
Feed conversion[1,2]	2.44[a]	2.41[a]	2.28[ab]	2.04[b]	2.14[ab]	2.01[b]	2.16[ab]

[1] Kg of feed consumed per kg of bodyweight gain.

[2] Means sharing a common superscript are not significantly ($P < 0.05$) different from each other.

ADG, feed consumption, or FC, although ADG and feed consumption were 60 g and 160 g/day lower, respectively, when Diet 2A was fed. Replacement of the removed protein N with urea N similarly was without effect. However, when L-lysine was added to Diet 2A, (Diet 4A), FC was improved ($P < 0.05$) while ADG and feed consumption were equivalent to performance of pigs fed the basal diet. When 2% urea was added to Diet 4A (to produce Diet 5A), ADG, feed intake, and FC were not significantly affected.

Subsequent additions of L-lysine in combination with DL-methionine, with or without urea, did not result in improvements in performance beyond those achieved when L-lysine was added alone.

The results of Experiment 3 suggest that the pig was able to meet its N requirement from the reduced level of protein contained in Diet 2A; however, addition of L-lysine to the low protein diet increased the efficiency of N use as evidenced by improved ($P < 0.05$) FC. It would, therefore, appear that protein was not restricted sufficiently, in either of Experiments 1, 2, or 3 to promote the utilization of dietary urea N. These results confirm those of Bowland (1967).

Experiment 4 -- Response of growing and finishing swine to urea, L-lysine, and DL-methionine

Factor means of the growth and carcass data of Experiment 4 are shown in Table 5. Corresponding mean squares obtained by analysis of variance of the group means are shown in Table (iv) of the Appendix.

Dietary treatment means are shown in Table 6. Interactive effects of urea and amino acids are shown in Table 6A.

Growth data

Two percent urea substituted for an isonitrogenous quantity of fish-

meal and soybean meal in 15.5% protein diets reduced ($P < 0.05$) ADG and feed consumption and caused a non-significant increase of 11% in FC. Kornegay et al. (1965b) reported that 44 kg pigs receiving 2.5% urea, either added to, or isonitrogenously replacing protein, in 17% protein diets, exhibited reduced ADG and poorer FC.

Although the influence of amino acid supplementation was not significant, additions of L-lysine, or a combination of L-lysine and DL-methionine, tended to increase ADG, feed consumption, and reduce FC. Comparison of treatment means (Table 6) shows that the deleterious effects of urea on ADG and FC tended to be overcome by additions of 0.42% L-lysine. That is, performance of pigs fed Diet 4B was not significantly different from those fed basal Diet 1B, but was significantly ($P < 0.05$) improved as compared to the performance of pigs fed Diet 2B. Additions of 0.21% L-lysine, with or without 0.09% DL-methionine, did not result in any improvements in performance, as compared to the pigs fed the basal diet, indicating adequacy of the basal diet with respect to these essential amino acids. Additions of 0.42% L-lysine in combination with 0.09% DL-methionine to the urea supplemented diet (2B) did not significantly affect ADG.

Feed consumption followed a pattern similar to that established by ADG; also, FC tended to be deleteriously affected on the inclusion of urea in the diet. Amino acid additions, however, tended to mask the inhibitory effects of urea on FC.

A significant ($P < 0.01$) urea by amino acid interaction, as illustrated by the treatment means, influenced ADG and feed consumption of pigs in Experiment 4. Illustration of the data in a two-way classification

Table 5. Experiment 4 -- Factor means of daily feed consumption, daily gain, feed conversion, and carcass measurements

Factor	Growth Data				Carcass Data				
	No. of Pigs	Daily Gain kg	Daily Feed kg	Feed Conversion kg/kg gain	No. of Pigs	Loin Area $(cm)^2$	Total Backfat[1] cm	Lean of Hamface %	R.O.P. Score %
Urea (%)		*	*			**		**	*
0	16	0.76	2.27	3.01	14	25.05	10.92	42.68	76.89
2	17	0.67	2.18	3.34	16	20.08	11.89	40.07	74.81
Amino acids									
0	10	0.67	2.17	3.36	9	22.10	10.72	43.39	76.87
L-lysine	11	0.71	2.24	3.15	11	22.63	11.61	42.74	75.65
L-lysine and DL-methionine	12	0.75	2.27	3.01	10	23.02	11.86	43.40	75.02
Sex		**	**			*		*	**
Barrows	12	0.77	2.39	3.11	10	21.19	11.61	41.26	75.01
Gilts	21	0.65	2.07	3.24	20	23.87	11.18	45.10	76.69

[1] Total of three measurements.

* Following means are significantly different at $P < 0.05$; ** are significantly different at $P < 0.01$

Table 6. Experiment 4.-- Dietary treatment means of daily feed, daily gain, and feed conversion

	Diet Number					
	1B	2B	3B	4B	5B	6B
N x 6.25 (%)	15.3	15.5	14.9	15.2	15.0	15.3
Urea (%)	0	2.0	0	2.0	0	2.0
L-lysine (%)	0	0	0.21	0.42	0.21	0.42
DL-methionine (%)	0	0	0	0	0.09	0.09
Daily gain (kg)[1]	0.80[a]	0.54[b]	0.71[a]	0.72[a]	0.76[a]	0.75[a]
Daily feed (kg)[1]	2.34[a]	2.00[b]	2.23[a]	2.26[a]	2.24[a]	2.29[a]
Feed conversion[2]	2.92	3.70	3.14	3.14	2.95	3.05

1 Means with a common superscript are not significantly ($P < 0.05$) different from each other.

2 Kg of feed consumed per kg of bodyweight gain.

(Table 6A) serves to clarify the interactive effects of urea and amino acid combinations on ADG and feed consumption.

These results essentially agree with those of Experiments 1, 2, and 3 as well as with the results of Hays et al. (1957), which indicated that L-lysine and DL-methionine additions to urea diets of 32.7 kg pigs did not improve ADG or FC. Kornegay et al. (1966) observed a reduction in the ADG and FC of pigs fed corn-soybean meal diets containing urea when 0.50% L-lysine was added. The depression in growth caused by L-lysine was overcome through supplementation of the diets with DL-methionine and

Table 6A. Experiment 4 -- Effects of urea and amino acids on daily gain and daily feed

Criterion[1]	Urea %	Amino Acid Combination			
		0	L	L & M	Mean
Daily gain	0	0.80[a]	0.71[a]	0.76[a]	0.76
	2	0.54[b]	0.72[a]	0.75[a]	0.67
Mean		0.67	0.72	0.76	
Daily feed	0	2.34[a]	2.23[a]	2.24[a]	2.27
	2	2.00[b]	2.26[a]	2.29[a]	2.18
Mean		2.17	2.24	2.27	

[1] Means with a common superscript are not significantly ($P < 0.05$) different from each other

tryptophan.

Barrows grew faster ($P < 0.01$) and consumed more feed ($P < 0.01$) than did gilts, although FC was not significantly different. Bowland (1967a) reported that self-fed finishing barrows generally consumed more feed and exhibited higher ADG than gilts.

Carcass data

Inclusion of urea in the diet decreased ($P < 0.01$) the area of longissimus dorsi (loin area), resulted in reductions ($P < 0.01$) in lean as a percentage of the ham face, and lowered ($P < 0.05$) the R.O.P. score. Reduced loin area, reduced lean in the ham face, a tendency towards more

total backfat, and reduction of R.O.P. score indicate that the urea-fed pigs deposited more fat. Presumably, this was the result of a restriction of dietary protein in relation to energy. In general, high protein diets result in leaner carcasses at a given level of digestible energy intake (Seerley, Polley, and Wahlstrom, 1964; Lee, McBee, and Horvath, 1967). It is possible, therefore, that protein was restricted and urea was not able to meet the N demand of the animal.

Amino acid additions in the form of L-lysine, or a combination of L-lysine plus DL-methionine, did not significantly affect carcass quality. However, R.O.P. score tended to decrease when either L-lysine, or L-lysine plus DL-methionine in combination, was added. This suggests that crystalline amino acids added to practical diets may result in an amino acid imbalance and thereby affect performance deleteriously.

Barrows had less ($P < 0.05$) loin area, reduced ($P < 0.05$) lean as a percentage of the ham face, and lower ($P < 0.01$) R.O.P. score than did gilts. These results agree with those reported by Bowland and Berg (1959). Robinson and Lewis (1964) and Robinson (1965) reported that the female carcass was, in general, superior in all respects. Total backfat of barrows tended to be greater than the total backfat of gilts but the difference was not significant. Backfat of barrows, however, has been reported to be greater than that of gilts in most cases (Wagner et al., 1963; Hale and Southwell, 1967; Young et al., 1968).

Urea depressed performance as in earlier experiments in that ADG was reduced ($P < 0.05$), feed consumption was reduced ($P < 0.05$), and FC increased by 11% (non-significant). Amino acid supplementation,

as L-lysine or DL-methionine, did not significantly influence growth or carcass quality. Amino acid additions masked the deleterious effects of urea in the diet but had no effect when added to the basal diet. Carcasses of urea-fed pigs were poorer than those not receiving dietary urea. Carcass quality was not influenced by supplemental amino acids but was influenced by sex.

Experiment 5 -- Metabolism of energy and nitrogen

Factor means of coefficients of N and energy metabolism are shown in Table 7, while Table 7A shows the influence of urea and amino acids on N digestibility. The corresponding mean square values obtained by analysis of variance are presented in Appendix Table (v). Figure 1 illustrates the utilization of N and energy.

Digestibility and retention of energy and nitrogen

Effects of age and bodyweight

Pigs of 65 kg bodyweight digested 4.7% less ($P < 0.01$) of their gross energy intake than the 25 kg pigs. Energy retained as a percentage of the gross energy consumed was similarly affected; the 65 kg pigs retained 3.2% less ($P < 0.01$) of the gross energy intake than was retained by the 25 kg pigs. However, pigs of 65 kg bodyweight retained more ($P < 0.01$) of the digestible energy than did the 25 kg pigs. In contrast to these results, Kuryvial (1961) and Lloyd, Crampton, and MacKay (1957) reported that as weight and age increase, the percentage apparent digestibility of energy tends to increase. An increased coefficient of energy retained as a percentage of digestible suggests that while digestible energy was lower in the heavier pigs, compensation was made for this by making more efficient

use of the energy digested.

Digestibility of N was lower ($P < 0.05$) in the 65 kg pigs. Although the coefficient of N digestibility was lower, N retention was not influenced and tended to remain constant at 16 to 17 g of N per day (Figure 1) for both weight groups. Other workers (Hays et al., 1959; Long et al., 1962) have reported that N retention decreases as age and weight increase. However, Oslage and Fliegel (1965) found that N retention for pigs from 20 to 110 kg remained relatively constant at 16.5 to 18.5 g/day although retention as a percentage of digestible N decreased from 60 to 35% over the period.

Effects of 2% dietary urea

Urea in the diet did not significantly affect energy digestibility or retention; however, the coefficient of N digestibility was higher ($P < 0.01$) when urea was included in the diet. Bowland (1967) observed similar results in earlier trials at this university. Visek (1964) reported that urea of endogenous or exogenous origin can freely diffuse across intestinal membranes. Increased N digestibility of diets containing urea would, therefore, be anticipated since absorption of urea from the gut may possibly be restricted to a lesser extent than absorption of protein N.

Coefficients of retention of gross and retention of digestible N were not significantly affected by 2% dietary urea. Hays et al. (1957), however, reported that decreases in N retention by 37 kg pigs were linearly related to increasing urea levels in the diet.

Effects of amino acids

Supplementation with L-lysine, or L-lysine and DL-methionine, did

Table 7. Experiment 5 -- Energy and nitrogen metabolism of pigs 25 and 65 kg bodyweight

Factor	No. of Pigs	Digestibility		Retention of Gross[1,2]		Retention of Digestible[1,2]	
		Energy %	Nitrogen %	Energy %	Nitrogen %	Energy %	Nitrogen %
Weight (kg)		**	*	**		**	
25	24	87.1	85.7	83.1	35.1	95.4	41.0
65	24	82.3	84.1	79.9	37.2	97.0	44.4
Urea (%)			**				
0	24	84.7	83.6	81.6	36.0	96.3	43.1
2	24	84.7	86.2	81.4	36.4	96.2	42.2
Amino acids							
0	16	84.4	85.4	81.4	39.8[a]	96.5	46.6[a]
L-lysine	16	85.1	85.3	81.9	36.1[ab]	96.2	42.3[ab]
L-lysine and DL-methionine	16	84.5	84.0	81.1	32.8[b]	96.0	39.2[b]
Sex		**		**	*		
Barrows	24	83.9	84.5	80.7	34.4	96.2	40.7
Gilts	24	85.4	85.3	82.2	38.0	96.3	44.6

[1] Means sharing a common superscript are not significantly ($P < 0.05$) different.

*Following means are significantly ($P < 0.05$) different.

**Following means are significantly ($P < 0.01$) different.

[2] Energy retention refers to metabolizable energy divided by either gross energy or digestible energy.

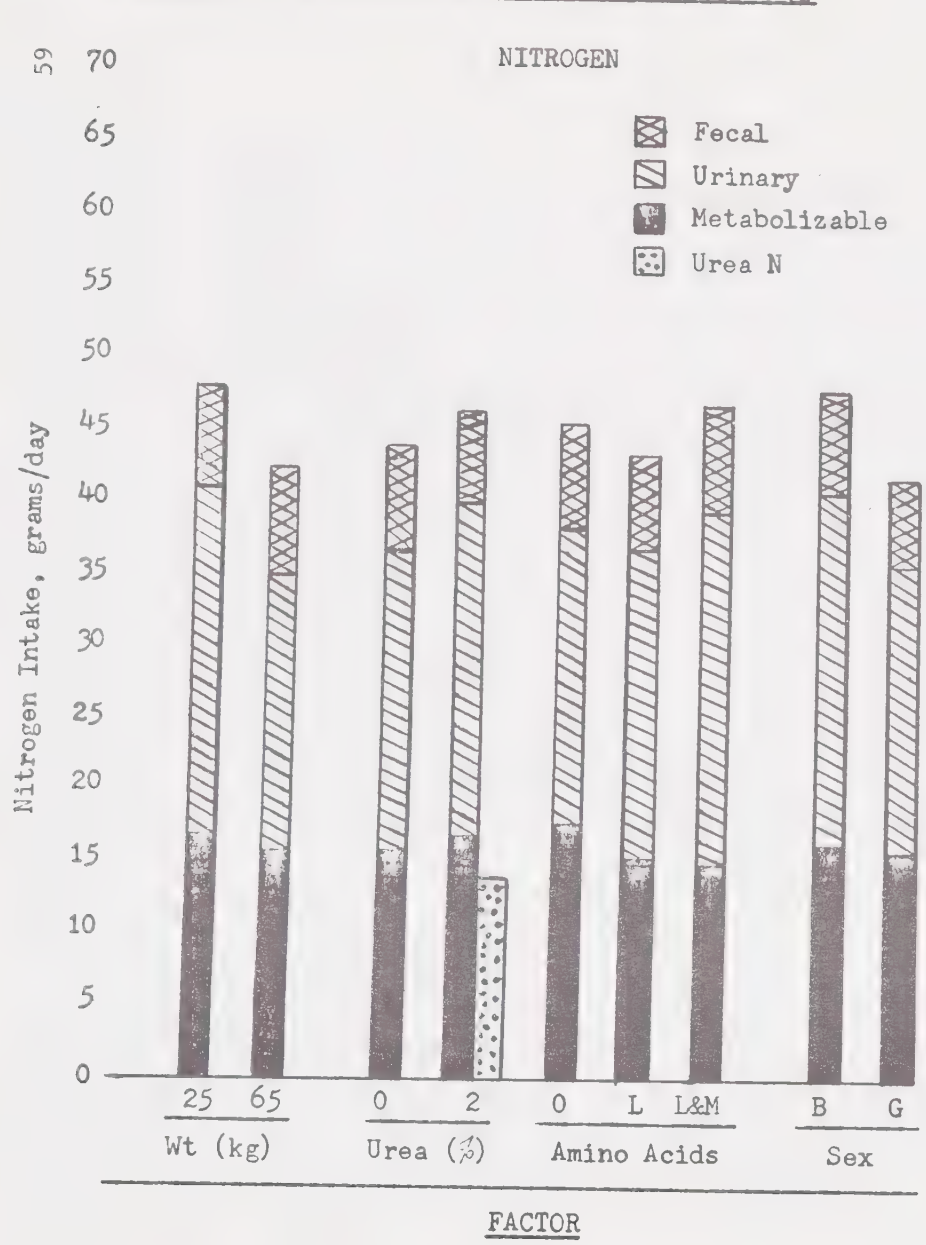
not affect the coefficients of digestibility of energy, digestibility of N, or energy retention. The N retained, either as a percentage of gross intake or as a percentage of digestible N, was lower ($P < 0.05$) when L-lysine and DL-methionine were added in combination. L-lysine alone tended to reduce N retention although not significantly. In contrast, Anderson and Bowland (1967) reported significant improve- in N retention as lysine was increased in the diet, indicating that improved amino acid balance increased N retention. It would, therefore, appear that the additions of L-lysine and DL-methionine in these experiments caused an amino acid imbalance as evidenced by poorer N retention of gross and digestible N.

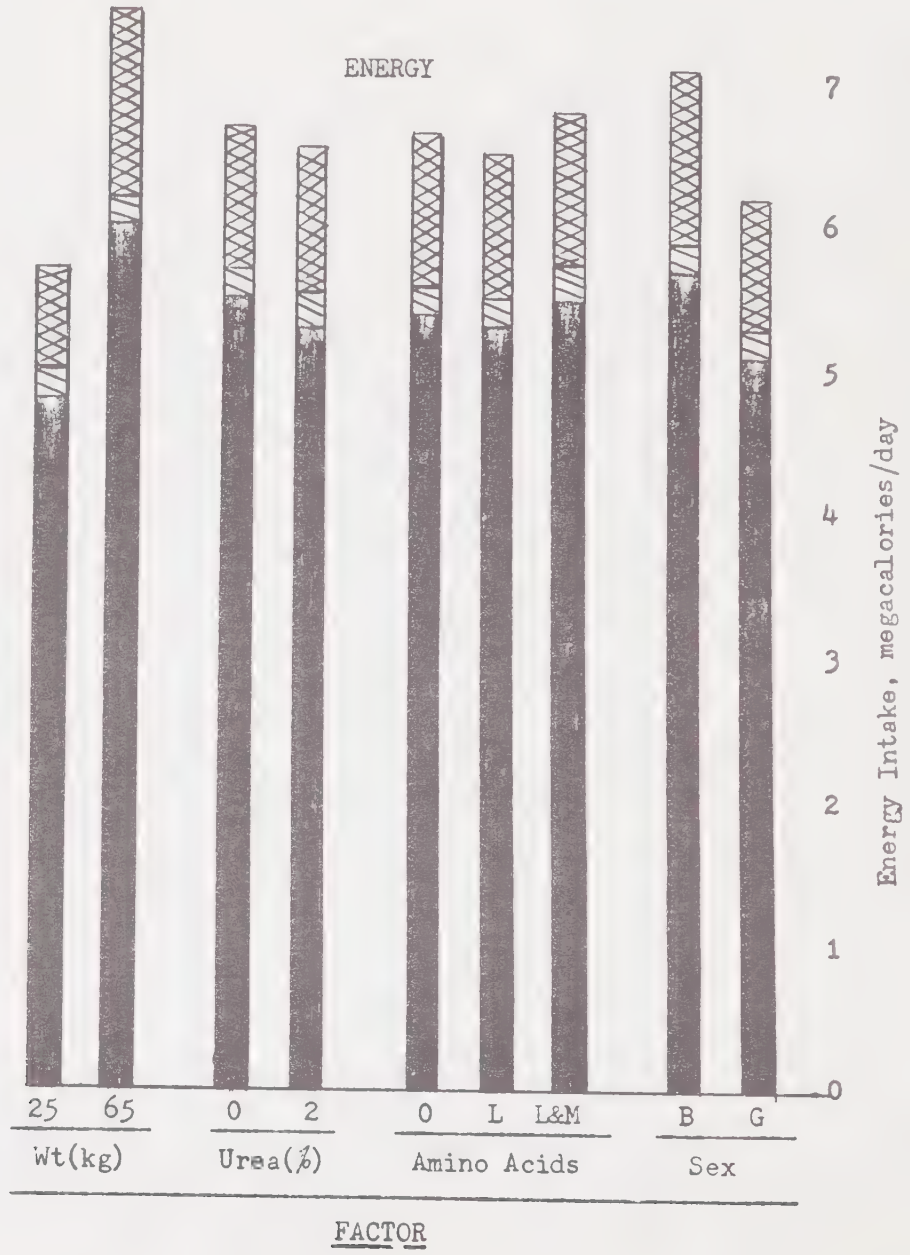
A significant ($P < 0.01$) interaction of weight and amino acids influenced the digestibility of N. As shown in Table 7A, while amino acid additions did not appreciably influence the coefficient of N

Table 7A. Experiment 5 -- Effects of weight and amino acids on the digestibility of nitrogen

	Amino Acid Combination			
Weight (kg)	0	L	L & M	Mean
25	87.8	85.2	86.1	85.7
65	85.0	85.4	81.9	84.1
Mean	85.4	85.3	84.0	

Figure 1. Utilization of nitrogen and energy





digestibility of the younger pigs, additions of L-lysine in combination with DL-methionine resulted in a lower coefficient of N digestibility in the older group.

These data tend to support the hypothesis that methionine was not the second most-limiting amino acid in these diets. Furthermore, the amino acid imbalance appeared to become more pronounced as the pigs got heavier. Müller and Málek (1967) have suggested that diets consisting entirely of a cereal grain may be more deficient with respect to threonine and tryptophan than methionine.

Effects of sex

Gilts were more efficient ($P < 0.01$) than barrows in terms of energy digested and retained as percentages of their gross energy intake. Retention of energy as a percentage of digestible energy was not, however, significantly different between gilts and barrows.

Gilts tended to exhibit higher coefficients of N digestibility although the difference was not significant. Also, gilts retained more of their gross N intake ($P < 0.05$) and more of the digestible N (non-significant) than barrows. However, N retained by either gilts or barrows was about 16 g of N per day (Figure 1), indicating that although differences existed in the efficiency of N use, absolute retention values tended to remain relatively constant between the sexes.

Relationships of energy and nitrogen retention

The ratios of energy retained (kcal/day) to N retained (g/day), the ME/MN, for the 25 kg and 65 kg pigs were 282 and 375, respectively. The widening ratio with increasing weight is indicative of an increasing tendency to deposit fat, resulting in a decreasing N requirement relative

to the energy requirement of the pig. This agrees with data published in The Nutrient Requirements of Farm Livestock--Agricultural Research Council (1967).

Urea addition at a level of 2% resulted in reduction of ME/MN from 344 (without urea) to 312 (with 2% urea). This would suggest that urea improved N retention in relation to metabolizable energy.

Widening ME/MN ratios (300, 331, and 367, respectively) as amino acids were added suggest that the additions of L-lysine, or L-lysine in combination with DL-methionine, resulted in reduced efficiency of N fixation in body tissues; ME, however, tended to remain relatively constant (5300 to 5500 kcal/day--Figure 1) so that MN decreased not only in absolute terms but also in relation to ME.

Barrows tended to retain more energy in relation to N retained than gilts as evidenced by respective ME/MN ratios of 346 and 319. This agrees with the results of the carcass study (Experiment 4) which showed that barrows deposited more fat in the carcass than gilts.

General observations

Digestible energy intake (Figure 1) of the pigs in the metabolism experiment was lower than suggested requirements (NRC, 1968). Average digestible energy consumed ad libitum by the 25 and 65 pigs was 5000 and 6200 kcal/day, respectively, as compared to the suggested requirements of 5610 and 10,230 kcal/day. Although the 25 kg pigs consumed nearly as much digestible energy as the published requirements suggest, consumption of digestible energy by the 65 kg pigs was 4030 kcal/day lower than published requirements. Similarly, crude protein intake of the larger pigs tended to be lower than suggested levels.

Presumably, greater stress was placed on the larger animals than the smaller animals when they were confined to the metabolism cages. It was noted during the course of the collection period that the feed consumption of the 65 kg pigs was low regardless of inclusion of urea in the diet. However, coefficients of energy digestibility and retention were comparable to those reported by Kuryvial (1961) and Schuld (1967), suggesting that although feed intake was low, the coefficients of energy and N metabolism were not affected.

In summary, although urea did not influence the retention of N, addition of 2% urea in the diet caused an increase ($P < 0.01$) in N digestibility. Although urinary N could account for all of the dietary urea N (Figure 1), it would appear that some urea N was retained in the body. Additions of amino acids to the diet caused a decrease ($P < 0.05$) in the coefficient of N retention (either gross or digestible N) indicative of an amino acid imbalance. Both weight and sex had important influences on the energy and N metabolism of the pigs in Experiment 5.

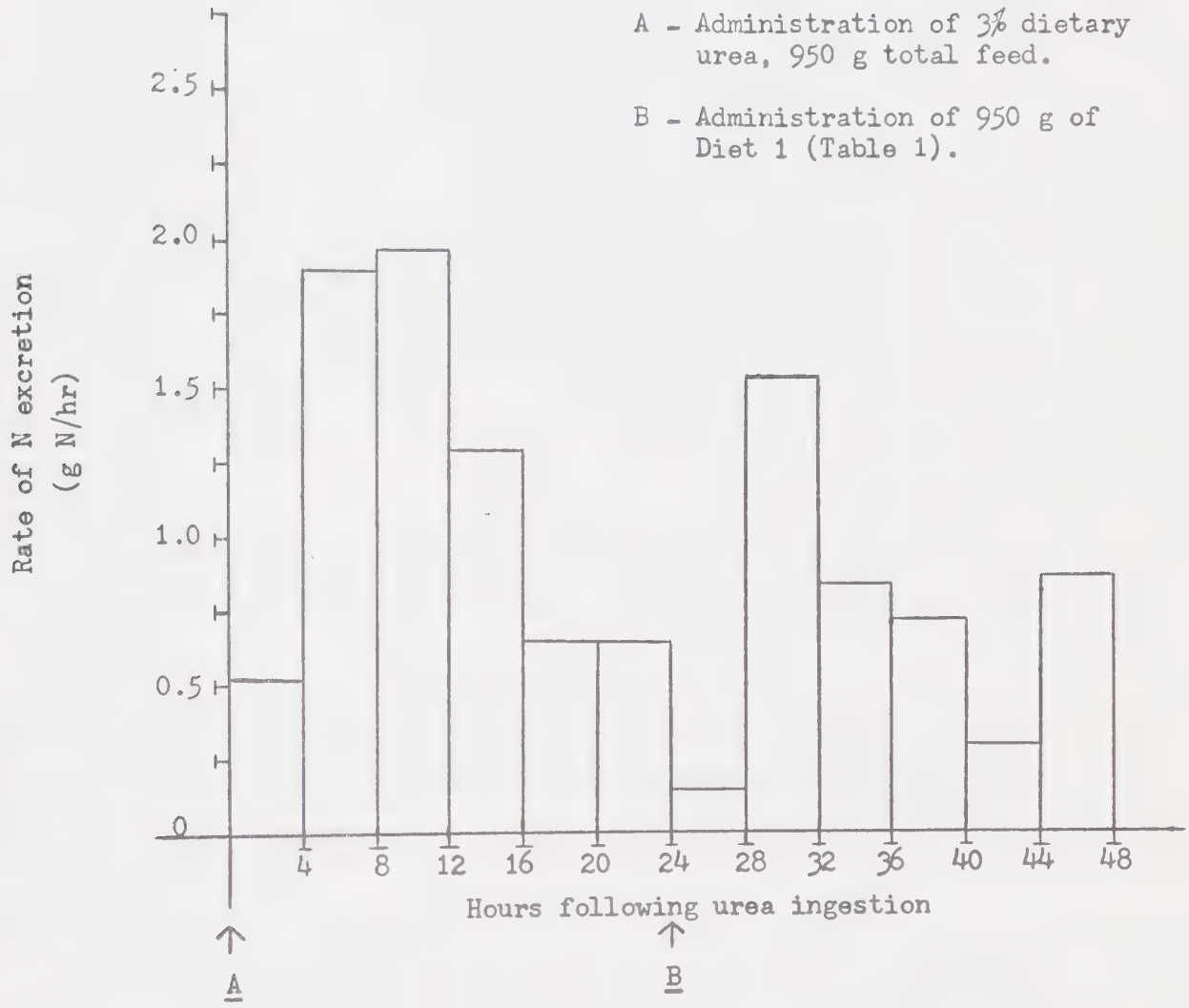
Experiment 6 -- Preliminary study: nitrogen excretion pattern

Figure 2 shows the average rate of urinary N excretion of three 22.7 kg gilts following the ingestion of 950 g of a mixture containing 97% Diet 1 and 3% urea.

The rate of N excretion via the urine increased rapidly from 4 to 8 hr following ingestion of the mixture containing 3% urea. A maximum rate of excretion of approximately 2 g N/hr occurred during a 4 to 12 hr period after the urea was consumed by the pigs.

Maximum N clearance (g N/hr) via the urine following a single

Figure 2. Average[1] rate of N clearance via the urine following ingestion of 3% urea in addition to normal protein



1 Average of three pigs.

feeding of 950 g of Diet 1 (without urea) was 1.55 g N/hr, or appreciably less than that observed from a diet containing 3% urea. A similar pattern of N clearance to that resulting from inclusion of 3% urea in the feed was obtained, however, in that the maximum rate of N excretion occurred 4 hr following feeding.

Total N consumption of 42.8 g of N included 12.8 g N from urea during period A (Figure 2); N supplied by 950 g of basal Diet 1 (period B) was 31.9 g. Urinary clearance of N during the 24 hr following the feeding of urea (period A) was 27.9 g, or 10.1 g more than the total N excreted in the urine during the subsequent 24 hr (period B) following the ingestion of a diet without urea. Increased urinary N excretion during the 24 hr following ingestion of urea, therefore, accounted for an average of 79% of the urea that was fed to the three pigs.

On the basis of the results of the preliminary study, it was possible to estimate the period of time that was necessary for urea, or NH_3 resulting from ureolysis, to reach maximal levels in the blood. Based on estimates of protein turnover rates of various tissues (Sprinson and Rittenberg, 1949b), it was estimated that maximum incorporation of urea N into tissue protein would not occur until 72 hr following peak N clearance in the urine.

In view of these results, therefore, the animals in Experiment 7 were killed 96 hr following the administration of N^{15}-urea in the diet.

Experiment 7 -- The fate of dietary N^{15}-urea in 10 kg barrows

The concentration of N^{15} in urine, feces, and trichloroacetic acid precipitates of body tissue and blood is presented in Table 8.

The N^{15} concentration of the diet as determined by calculation and by analysis is shown in Table 9. The total N^{15} content of urine, feces, and liver and estimated total N^{15} content of blood and skeletal muscle, as a percentage of the administered dose, are presented in Table 10.

The N^{15} content of blood, tissue, and excreta

Dietary treatment did not exert any influence on the degree to which N^{15} was incorporated into the tissues; also, excretion of N^{15} label in the urine and feces was not significantly affected. However, plasma trichloroacetic acid precipitate N^{15} concentrations of the pigs receiving supplemental L-lysine and DL-methionine were greater ($P < 0.05$) than those of pigs receiving Diet 2 containing N^{15}-urea exclusive of supplemental L-lysine and DL-methionine. Since significant differences were not detected between dietary treatments of the remaining fractions, average values for the four pigs are discussed.

Average urinary N^{15} content during the first 48 hr following N^{15}-urea administration was 2.428 atom % excess. Subsequent urinary N^{15} content 48 to 96 hr later was 0.063 atom % excess. These results indicate that the major period of N^{15} excretion occurred during a relatively short period following ingestion.

Fecal N^{15} content was 0.130 atom % excess, accounting for only a small fraction of the total N^{15} relative to that of the urine. This suggests that urea tends to be readily absorbed from the intestine, as reported by Visek (1964).

The incorporation of N^{15} into tissue trichloroacetic acid precipitates was highest in that of the liver. This fraction contained 0.033

Table 8. Experiment 7 -- N^{15} concentrations[1] in urine, feces, and tissue trichloroacetic acid precipitates

	Urea		Urea plus Amino Acids		Avg of
	Pig 2A	Pig 2B	Pig 6A	Pig 6B	4 Pigs
	Atom % Excess	Atom % Excess	Atom % Excess	Atom % Excess	Atom % Excess
Urine					
0 to 48 hr	2.588	2.295	2.469	2.361	2.428
48 to 96 hr	0.050	0.100	0.052	0.049	0.063
Feces					
0 to 96 hr	0.100	0.204	0.130	0.083	0.130
Trichloroacetic acid precipitates					
Liver	0.032	0.036	0.034	0.032	0.033
Plasma	0.027	0.027	0.029	0.029	0.028
Blood cells	0.003	0.003	0.002	0.005	0.003
Intestinal scrapings	0.024	0.027	0.018	0.027	0.024
Gluteus maximus	0.010	0.008	0.008	0.012	0.009
Longissimus dorsi	0.007	0.009	0.009	0.010	0.008

[1] A blank urine sample contained 0.3508 atom % N^{15}.

atom % excess. Liu et al. (1955), in studies with pigs of similar size to those used in this experiment, reported that liver protein tended to be more highly enriched than kidney, blood, or ham muscle protein. These values were recorded by Liu et al. (1955) three days following administration of 1800 mg of N^{15}-urea (15.06 atom % excess). Sprinson and Rittenberg (1949b) estimated that one-half of the synthesis of protein in a rat was associated with liver and serum protein. Therefore, enrichment of these fractions would be expected to be highest.

Generally lower values for N^{15}-incorporation were obtained by Liu et al. (1955) than were obtained in the present study. These workers reported that 0.02 atom % excess of N^{15} was incorporated into liver protein of pigs following administration of approximately one-quarter of the dose of N^{15} that was administered in these experiments. Lower N^{15} incorporation may have also resulted from the fact that the pigs used in their experiments were not allowed a period of adjustment to dietary urea prior to administration of N^{15}-urea. Therefore, more N^{15} of administered N^{15}-urea may have been utilized in protein synthesis by the pigs in our experiments since a 14 day period of adjustment to urea preceded administration of N^{15}-urea. Kornegay et al. (1965b) reported that the initial depressant effects of urea tended to disappear as the pigs adjusted to its inclusion in the diet.

Blood plasma and scrapings of the intestinal mucosa had N^{15} concentrations of 0.028 and 0.024 atom % excess, respectively. Presumably, amino acids synthesized in the liver are used *a priori* for plasma protein synthesis. This is proposed since enrichment of the liver and blood plasma fractions were similar. Similarity of blood

and liver protein enrichment following administration of N^{15}-urea has been reported in pigs (Liu et al., 1955), rats (Kornberg and Davies, 1952), and mice (Bloch, 1946).

Enrichment of the intestinal mucosa reflects a high protein turnover rate, only slightly less than that of the plasma trichloroacetic acid-insoluble fraction. The intimate contact of the blood and intestinal surface appears to provide an efficient means of amino acid transfer to the inner lining of the gut to replenish the rapidly degraded, proteinaceous, mucosal layer.

Trichloroacetic acid precipitates of blood cells contained an average N^{15} content of 0.003 atom % excess, or approximately 12% of the level of enrichment of the plasma fraction. Relative to plasma protein, blood cell protein, therefore, appears to be synthesized at a much lower rate. These results essentially agree with results reported by Snyderman et al. (1962) which indicated that N^{15} comprised 4.9 and 13.0% of the N of blood cell and plasma protein synthesized during the administration of N^{15}-urea to human subjects.

Skeletal muscle contained the least N^{15} as illustrated by the low enrichment of the trichloroacetic acid precipitates of the longissimus dorsi and gluteus maximus muscles. These fractions contained similar N^{15} concentrations of 0.008 and 0.009 atom % excess, respectively. The protein half-life of skeletal muscle in rats was estimated by Sprinson and Rittenberg (1949b) to be in the order of 28 days, or considerably longer than the rate of protein turnover of liver or blood. Hence, considerably lower N^{15} enrichment values would be expected in skeletal muscle protein. Liu et al. (1955) also observed

that the protein fraction of the ham muscle of pigs contained the lowest concentration of N^{15} relative to liver, kidney, and blood protein fractions.

Although not clearly illustrated by the preceding growth and metabolism experiments, it is apparent from the results of Experiment 7 that dietary N^{15}-urea did supply some of the nitrogen used in body protein synthesis. These data support the results, moreover, of earlier trials with other monogastric species where N^{15}-urea was administered (Bloch, 1946; Kornberg and Davies, 1952; Liu et al., 1955).

Analysis of the feed containing N^{15}-urea (Table 9) was carried out as confirmation of the level of N^{15} that was administered. By analysis, feed 2 contained 11.51 atom % excess N^{15}; feed 6 contained 11.09 atom % excess N^{15}. These concentrations of N^{15} were equivalent to 561 and 548 mg of N^{15}, respectively, and account for an average of 94% of the calculated administered dosage of 591.6 mg of N^{15}. Close

Table 9. Experiment 7 -- Level of N^{15} administration by calculation and by analysis

Administered in Feed	Diet 2	Diet 6
N^{15}-urea (g)	1.25	1.25
N^{15} calculated (mg)	591.6	591.6
N^{15} analysis of feed		
Atom % excess	11.51	11.09
N^{15} (mg)	561.1	548.4

agreement of the N^{15} content of the feed by analysis to the calculated value indicates that the N^{15} dosage was near expectation. Percentage recovery values (Table 10) were calculated using the value of 591.6 mg of N^{15}.

Recovery of N^{15}

Average urinary excretion of N^{15} (Table 10) accounted for 52.2% of the administered N^{15} during the 48 hr period following the ingestion of the N^{15}-urea. Pig 6B during this time produced less urine than the remaining three pigs; also, urine N^{15} concentrations of pig 6B tended to be lower. No obvious reason can be found for the lowered N^{15} excretion by this pig, other than individual variation.

Urinary excretion of N^{15} during the subsequent 48 hr period accounted for an average 1.9% of the administered label. This agrees with the results of experiments with rats (Kornberg and Davies, 1952) injected with N^{15}-ammonium lactate. Excretion of N^{15} via the urine of rats injected with N^{15}-urea, furthermore, indicated that approximately 84% of the injected isotope was cleared 40 to 48 hr post-administration. Liu et al. (1955) reported a similar rate of N^{15} clearance in the urine of pigs which were administered with N^{15}-urea in capsulated form over a period of three days.

Fecal N^{15} clearance accounted for only 1.3% of the administered dosage during the 96 hr experimental period. Since the diet contained antibiotic feed supplement (Table 1), bacterial protein synthesis was probably minimal (Visek et al., 1965) which could account, in part, for low fecal N^{15} output. Similarly, urea being a readily diffusible compound, would tend to be absorbed from the intestine with relative

Table 10. Experiment 7 -- N^{15} excretion and retention as a percentage of the administered dosage

	Urea (Diet #2)		Urea plus Amino Acids (Diet #6)	
	Pig 2A %	Pig 2B %	Pig 6A %	Pig 6B %
Excretion				
Urine				
0 to 48 hr	55.34	54.23	60.52	38.83
48 to 96 hr	1.32	3.45	1.40	1.29
Total	56.66	57.68	61.92	40.12
Feces 0 to 96 hr	0.93	1.78	1.34	0.95
Incorporation				
Liver protein	0.34	0.41	0.39	0.44
Plasma protein*	0.22	0.21	0.24	0.25
Blood cell protein*	0.02	0.03	0.02	0.04
Dorsal muscle protein*	0.60	0.70	0.70	0.80
Pelvic limb protein*	1.60	1.10	1.30	1.90
Other muscle protein*	1.40	1.30	1.40	1.90
Total recovered	61.77	63.21	67.31	46.40

* Protein contributions to the weight of the pig are calculated from data of Kauffman and St. Clair (1965) and ARC (1967).

ease as compared to N of protein origin.

Calculations of the quantity of N^{15} incorporated into the various protein fractions were based on values reported by Wood (cited by ARC, 1967) and Kauffman and St. Clair (1965). Protein of the empty liveweight of the pig was calculated by using a factor of 14.5% (ARC, 1967). The relative nitrogen distribution of the protein in the body of each pig was derived by application of values reported by Kauffman and St. Clair (1965). Total blood volume was estimated as being 8% of the empty liveweight (Freidman, 1966).

Average N^{15} incorporation into the liver by the four pigs accounted for 0.4% of the administered N^{15} label. Liver protein contributes less to the total liveweight of the pig than does total skeletal muscle. Therefore, less N^{15}, as a percentage of the administered dose, is used in liver protein synthesis than in muscle protein synthesis, even though the concentration of N^{15} in the liver was considerably higher than in skeletal muscle (Table 8).

Total N^{15} recovered, including the estimated percentages of the administered N^{15} present in blood and skeletal muscle fractions, accounted for an average of 60% of the administered label. Greater recovery of N^{15} may have been possible had N^{15} analysis been conducted on the trichloroacetic acid-soluble N compounds of blood and tissue fractions. Since the tissue and blood fractions were treated with cold, and subsequently hot trichloroacetic acid, NPN, free amino N, short chain peptides, and nucleic acid N were not analyzed for possible N^{15} enrichment. Presumably, greater knowledge of the fate of dietary urea would have been gained had these fractions been examined.

The results of Experiment 7, therefore, support the conclusion of Liu et al. (1955) that "...there is a small but definite amount of the administered urea incorporated into the body protein."

GENERAL DISCUSSION

Dietary urea generally caused depressions in the performance criteria of the pigs, both in the starting and growing-finishing stages. On the basis of rate of gain, feed conversion and carcass characteristics, urea did not appear to be of value in swine rations. Presumably, the protein-N content of the various diets containing urea was sufficient to support nearly normal growth, especially when the L-lysine content was increased. DL-methionine, however, did not appear to be a limiting amino acid since additional response did not occur when it was added to the diets in combination with L-lysine. Had protein-N been more restricted, it is possible that urea would have had some beneficial effects on pig performance.

Based on N metabolism studies, L-lysine and DL-methionine, added in combination, appeared to cause an amino acid imbalance. This apparent imbalance, characterized by reducing coefficients of N digestibility and retention, became more severe as the pigs grew older, as evidenced by the effects of a significant ($P<0.01$) amino acid x weight interaction on N digestibility. Carcass data tended to support this evidence of amino acid imbalance. Energy metabolism was not influenced by the inclusion of urea or of amino acids.

Results of the preliminary N excretion study (Experiment 6) suggest that when dietary protein was adequate to support normal development, dietary urea N was nearly quantitatively excreted during 24 hr following administration. However, urea isonitrogenously replacing protein in Experiment 5 increased the coefficient of digestible N significantly

($P<0.01$) without influence on the coefficient of N retained, suggesting that urea N was retained in the body when protein N was decreased. Conclusive evidence of the utilization of urea N for blood and body tissue protein synthesis was obtained from the N^{15}-urea study (Experiment 7). Relative differences in the N^{15} enrichment of the various fractions were considered to be reflective of the rate of protein synthesis in each case. The degree of N^{15} enrichment, however, could not be statistically related to the protein or amino acid status of the diet except in the case of the plasma fraction. Enhanced N^{15} incorporation may have resulted if the protein-N content of the diet had been more severely restricted.

Incorporation of N^{15} into the trichloroacetic acid-insoluble fractions, based on concentration per unit of weight, was greatest in liver, followed by blood plasma, intestinal scrapings, gluteus maximus, longissimus dorsi, and blood cells. Examination of trichloroacetic acid-soluble N probably would have allowed better documentation of the fate of N^{15}-urea.

Urinary excretion served to clear the major quantity of N^{15} of N^{15}-urea from the body of the pig during a relatively short interval of time following ingestion of the isotope. Fecal N^{15} output was insignificant relative to that excreted in the urine, probably due to the ease with which urea is absorbed from the intestinal tract. Inclusion of antibiotic feed supplement in the diet may have reduced not only bacterial utilization of urea for protein synthesis but also urea hydrolysis in the gut, thereby reducing the total quantity of NH_3 from urea that was available for protein synthesis by the animal.

GENERAL SUMMARY

Experiments were designed to investigate the effects of urea, lysine, and methionine additions to the diets of starting and growing-finishing swine.

Two percent urea isonitrogenously replacing fishmeal reduced ($P<0.01$) feed consumption, ADG, and FC in Experiment 1 but did not have any influence on these criteria in Experiments 2 and 3. In Experiment 3, failure of the low protein diet to significantly depress ADG, feed intake, and FC in relation to the high protein diet indicated that protein was not limiting and therefore the diets were not likely to facilitate the utilization of supplemental urea. In Experiment 4, dietary urea caused depressions ($P<0.01$) in feed intake and ADG but had no influence on FC. Reduced loin area ($P<0.01$), reduced lean in the ham face ($P<0.01$), and lower ($P<0.05$) R.O.P. score resulted from urea additions at a level of 2% of the diet. In metabolism studies, urea in the diet increased ($P<0.01$) the coefficient of N digestibility but did not influence N retention. Urea additions to the diet did not influence energy metabolism.

L-lysine (0.38%) addition to urea diets improved ($P<0.01$) FC of pigs fed diets containing urea in Experiment 1, but had no significant effect on ADG or feed consumption. Pigs in Experiment 2 did not show significant differences in performance resulting from L-lysine additions, although a trend similar to Experiment 1 was evident. L-lysine supplementation improved ($P<0.05$) FC of pigs receiving the low protein control diet such that it supported FC similar to that

supported by the 21.6% protein basal diet. However, ADG and feed intake were not influenced. Supplementation of the diets containing urea with L-lysine masked the deleterious effects caused by urea alone.

L-lysine in combination with DL-methionine, when added to the diets in Experiments 1, 2, or 3, did not result in any greater response than the response from L-lysine alone. A significant ($P<0.01$) urea by amino acid interaction in Experiment 4 indicated that while L-lysine improved ADG and FC of urea-fed pigs so that these became equivalent to the ADG and FC of pigs fed the basal diet, L-lysine and DL-methionine did not produce any greater response than L-lysine alone. Amino acid supplementation did not influence loin area, percent lean in the ham face, total backfat, or R.O.P. score in Experiment 4. Additions of L-lysine, or a combination of L-lysine and DL-methionine, did not significantly influence the coefficients of energy and N digestibility nor those of energy retention but did have a depressant effect ($P<0.01$) on N retention. An interaction ($P<0.01$) of weight by amino acid on the coefficient of N digestibility indicated that the 65 kg pigs were more severely affected by combined additions of the essential amino acids than were the 25 kg pigs.

Metabolism studies at two weights, 25 kg and 65 kg, showed that differences occurred in efficiency of N and energy utilization at these growth stages. The 25 kg pigs exhibited higher ($P<0.01$) coefficients of digestibility and retention of gross energy; however, the pigs of 65 kg bodyweight had higher ($P<0.01$) coefficients of energy retained of that which was digested. The coefficient of N digestibility was higher ($P<0.05$) for the 25 kg pigs than it was for the 65 kg pigs. However,

retention of N in absolute terms tended to be relatively constant between the two bodyweight groups.

Sex of the animal had no significant affect on ADG, feed intake, or FC in Experiments 1, 2, or 3. In Experiment 4, ADG and feed consumption of barrows were greater ($P<0.01$) than that of gilts; FC, however, was not significantly different between sexes. Barrows had less loin area ($P<0.05$), less lean in the ham face ($P<0.05$), and lower R.O.P. score ($P<0.01$) than gilts. Coefficients of energy digested and retained were higher ($P<0.01$) for gilts than for barrows. Gilts also retained more gross N ($P<0.05$) than the barrows.

Clearance of N in the urine accounted for 79% of the administered level of 3% urea during 24 hr following administration. A high rate of excretion of N in the urine was prolonged 4 hr by dietary urea, and the rate of N excretion was increased by 29%.

Excretion of N^{15} following administration of a single feeding containing 591.6 mg N^{15} from N^{15}-urea accounted for 52.2% of the administered label in the first 48 hr and 1.9% in the subsequent 48 hr. Fecal excretion of dietary N^{15} accounted for 1.3% of the administered dose.

Average values of N^{15} incorporation into trichloroacetic acid-insoluble fractions of tissues and blood, sampled 96 hr post-administration, was greatest in liver (0.033 atom % excess), blood plasma (0.028 atom % excess), and intestinal scrapings (0.024 atom % excess). N^{15} was incorporated to a lesser extent in the longissimus dorsi (0.008 atom % excess), gluteus maximus (0.009 atom % excess), and blood cells (0.003 atom % excess).

L-lysine and DL-methionine content of the diet did not have any

consistent influence on the utilization of N^{15} of N^{15}-urea in protein synthesis. However, a greater ($P<0.05$) amount of N^{15} was present in the trichloroacetic acid-insoluble fraction of the plasma of pigs which received supplemental L-lysine and DL-methionine, than in the plasma fraction of pigs fed the diet without supplemental amino acids.

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Table (i). Experiments 1 and 2 -- Mean squares obtained by analysis of variance of growth data

Source of Variation	Experiment 1 (4 to 10 weeks) Df	Daily Gain	Daily Feed	Feed Conversion	Experiment 2 (4 to 11 weeks) Df	Daily Gain	Daily Feed	Feed Conversion
Replicates	3	0.013	0.076	0.0175	-	---	---	----
Urea (U)	1	0.080**	0.097**	0.4816**	1	0.0004	0.0033	551.25
Amino acid (AA)	2	0.008	0.002	0.1562**	2	0.0017	0.0243	174.83
Sex (S)	1	0.010	0.016	0.0126	1	0.0008	0.0336	582.73
U x AA	2	0.004	0.002	0.0962*	2	0.0016	0.0172	205.04
U x S	1	0.007	0.016	0.0020	1	0.0004	0.0027	436.42
AA x S	2	0.001	0.000	0.0023	2	0.0040	0.0186	93.78
AA x U x S	2	0.002	0.001	0.0001	2	0.0010	0.0061	28.45
Error	33	0.003	0.011	0.0179	11	---	---	----
"Individual[1] error"					24	0.0014	0.0084	181.06
Total	47				35			

[1] Within treatment error mean square.

* Significant at $P < 0.05$; ** significant at $P < 0.01$.

Table (ii). Experiments 1 and 2 -- Mean squares obtained by analysis of variance in 2 x 6 factorial design

Source of Variation	Experiment 1				Experiment 2			
	Df	Daily Gain	Daily Feed	Feed Conversion	Df	Daily Gain	Daily Feed	Feed Conversion
Replicates	3	.013	.076	.018	--	--	--	--
Sex	1	.010*	.022	.013	1	.0008	.0036	582.73
Diets	5	.021**	.021	.197**	5	.0014	.0173	262.20
Sex x Diet	5	.001	.000	.001	5	.0021	.0104	136.17
Error	33	.003	.011	.018	--	--	--	--
Individual Error	--	--	--	--	24	.0014	.0084	181.06
Total	47				35			

*Significantly different at $P < 0.05$.
**Significantly different at $P < 0.01$.

Table (iii). Experiment 3 -- Mean squares obtained by analysis of variance of growth data

Source of variation	Df	Daily Gain	Daily Feed	Feed Conversion
Replicates	1	.0002	.0009	.0002
Sex	1	.0041	.0357	.0129
Diet	6	.0024	.0311	.1180*
Diet x Sex	6	.0026	.0357	.0867
Error	13	.0035	.0238	.0323
Total	27			

*Significantly different at $P < 0.05$.

Table (iv). Experiment 4 -- Mean squares obtained by analysis of variance of growth and carcass data

		Growth Data				Carcass Data			
	DF	Daily Gain	Daily Feed	Feed Conversion	DF	Loin Area	Total Backfat	Lean of Hamface	R.O.P. Score
Urea	1	0.0245*	0.0219*	0.3274	1	73.790**	2.587	115.63**	13.06*
Amino acids	2	0.0075	0.0095	0.1224	2	1.193	1.457	0.58	3.53
Sex	1	0.0432**	0.3062**	0.0472	1	22.814*	0.534	44.20*	8.47**
U x AA	2	0.0221**	0.0452**	0.2235	2	9.812	1.548	11.69	3.78
U x S	1	0.0000	0.0008	0.0075	1	0.188	0.037	16.12	0.14
AA x S	2	0.0012	0.0197	0.0017	2	1.163	0.824	7.18	1.42
Error	2	0.0026	0.0044	0.0928	1	1.193	0.653	8.13	1.69
Individual error	-	--	--	--	19	4.644	0.867	6.36	1.78
Total	11				29				

* Significant at $P < 0.05$.

** Significant at P 0.^1.

Table (v). Experiment 5 -- Mean squares obtained by analysis of variance of metabolism data

Factor	Df	Digestibility		Retention of Gross		Retention of Digestible	
		Energy	Nitrogen	Energy	Nitrogen	Energy	Nitrogen
Replicates	1	7.32	13.66	7.81	63.71	0.06	57.94
Weight	1	268.24**	30.77*	125.00**	56.07	29.13**	135.64
Urea	1	0.02	85.84**	0.31	1.99	0.27	8.99
Amino acids	2	2.10	9.85	2.46	195.52**	1.05	223.20*
Sex	1	25.80**	8.88	26.34**	161.99*	0.07	183.73
W x U	1	0.72	12.39	0.43	15.66	0.04	43.68
W x AA	2	0.34	21.30*	0.15	67.69	0.05	111.81
AA x U	2	2.94	13.50	2.03	72.12	0.48	113.64
S x W	1	8.76*	6.03	7.60**	104.02	0.02	116.10
S x U	1	0.03	1.78	0.13	26.64	0.34	32.36
S x AA	2	5.13*	0.80	6.80**	16.39	0.40	21.87
W x U x AA	2	1.52	2.33	2.14	5.68	0.35	10.62
W x U x S	1	0.47	4.27	2.20	31.17	0.93	28.04
W x AA x S	2	6.78**	2.20	7.34**	22.60	0.52	24.32
U x AA x S	2	0.28	7.36	0.41	70.09	0.05	104.77
U x W x AA x S	2	0.09	1.61	0.33	71.54	0.89	98.81
Error	23	1.13	4.26	0.96	32.91	0.33	48.77

* Significant at $P < 0.05$.

** Significant at $P < 0.01$.